

Abstract of

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(64) SYNTHESIS INHIBITOR CONTAINING GINSENOSIDE OF PROTEIN BELONGING TO HSP 27 FAMILY

(57) Abstract:

PROBLEM TO BE SOLVED: To obtain the subject HSP47 synthesis inhibitor capable of suppressing collagen synthesis and treating diseases showing the condition of sthenia of extracellular matrix production, comprising a saponoside as an active ingredient.

SOLUTION: Ginseng or koji is subjected to extraction treatment with water or an organic solvent and separated from impurities by filtration, centrifugal separation, etc., to give a crude extract, which is optionally purified by an adsorbent to give a ginsenoside (e.g. ginsenoside Rg1). Then, the ginsenoside is pharmaceutically manufactured alone or with an ordinary carrier to give the objective synthesis inhibitor which contains the ginsenoside as an active ingredient, has an inhibitory action on synthesis of a heat shock protein (HSP47) having 47 kilodalton molecular weight, suppresses collagen synthesis in organs and is useful for treating diseases such as cirrhosis, interstitial lung disease, chronic renal insufficiency, hypertrophy of heart, postoperative cicatrice, burn cicatrice, keloid, scleroderma, arteriosclerosis, arthrorheumatism, etc.

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(54)【発明の名称】 ジンセノサイド類含有HSP47合成抑制剤

(57)【要約】

【課題】 コラーゲン合成を抑制することによって、細胞外マトリックス産生の亢進の病態を示す病気を治療することができる、分子量47キログルトンの熱ショックタンパク質の合成抑制剤を提供する。

【解決手段】 ジンセノサイド類を有効成分として含有する。

【特許請求の範囲】

【請求項1】 ジンセノサイド類を有効成分として含有することを特徴とする、分子量47キログルトンの熱ショックタンパク質の合成抑制剤。

【請求項2】 ジンセノサイド類がジンセノサイドR_{g1}である、請求項1に記載の分子量47キログルトンの熱ショックタンパク質の合成抑制剤。

【請求項3】 ジンセノサイド類を含有する植物の抽出物を有効成分として含有することを特徴とする、分子量47キログルトンの熱ショックタンパク質の合成抑制剤。

【請求項4】 ニンジン又はコウジンの抽出物を有効成分として含有することを特徴とする、分子量47キログルトンの熱ショックタンパク質の合成抑制剤。

【発明の詳細な説明】

【0001】

【発明の属する技術分野】本発明は、ジンセノサイド類、特にジンセノサイドR_{g1}を有効成分として含有する、分子量が47キログルトン(kD)の熱ショックタンパク質(以下、HSP47と称する)の合成抑制剤に関する。本発明のHSP47合成抑制剤は、特に、臓器内のコラーゲンの合成を抑制することにより肝硬変、間質性肺疾患、慢性腎不全(又は慢性腎不全に陥る疾患)、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの細胞外マトリックス(細胞外基質)産生亢進の病態を示す病気の患者の生理学的状態を有効に改善させ、肝硬変、間質性肺疾患、慢性腎不全

(又は慢性腎不全に陥る疾患)、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの細胞外マトリックス産生亢進の病態を示す病気を効果的に治療することができる。

【0002】

【従来の技術】近年、コラーゲンなどの細胞外マトリックスの産生の亢進の病態を示す病気が大きな問題となっている。ここでいう細胞外マトリックス産生の亢進の病態を示す病気とは、例えば肝硬変、間質性肺疾患、慢性腎不全(又は慢性腎不全に陥る疾患)、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどを含む。

【0003】例えば、死亡者がわが国だけでも年間約2万人にものぼるといわれている肝硬変は、肝臓が結合組織の増殖のため固くなる病気の総称で、種々の慢性肝疾患の終末像であるといわれ、肝全体にわたるびまん性の肝線維症である。すなわち、炎症などの肝傷害が長期に及ぶ慢性肝炎においては、線維芽細胞や伊東細胞などの細胞外マトリックス(特にI型コラーゲン)産生の著しい亢進を伴い肝臓は線維化する。肝の線維化が慢性的に

進行すると、ますます正常な肝再生は妨害され、肝細胞に置き換わり、線維芽細胞とI型コラーゲンを主体とする細胞外マトリックスが肝組織のかなりの部分を占め、多くの微小葉からなる肝硬変に至る。肝硬変の進行に伴い、線維隔壁が肝全体に進展し、その結果生じる血流の異常は、肝実質細胞の変性を更に押し進める一因にもなり、肝硬変における惡循環が続くことになり、更にはアルコール、ウイルス、自己免疫等種々の原因によって、肝臓中に多量の膠原線維が生成され、肝細胞の壊死と機能消失とが生じ、肝硬変患者は遂には死に至る。I型コラーゲンは正常肝では全タンパク質量の約2%を占めるが、肝硬変となると10~30%を占めるようになる。

【0004】また、間質性肺疾患は、肺胞及び肺血管のみならず、しばしば呼吸細気管支や終末気管支も巻き込む下部気道の慢性炎症(肺胞炎alveolitis)とその結果である間質の線維化と肺胞内線維化を特徴とする疾患群である。ここでいう間質性肺疾患とは、例えば、間質性肺炎、肺線維症などのびまん性間質性肺疾患、特発性肺線維症、透過性肺水腫、膠原病肺、サルコイドーシスなどを含む。間質性肺疾患においては、線維化組織では細胞外マトリックスの過剰な産生と蓄積が認められている。すなわち、間質性肺疾患の肺線維化組織では、肥大した間質に著明なI型及びIII型コラーゲンの集積がみられており、特にIII型コラーゲンは、線維化の早期に肥厚した肺胞中隔に集積し、病期が進行し、後期にはI型コラーゲンが増加し、主要なコラーゲンとなる。基底膜は早期に破壊されており、肺胞腔側へのコラーゲン線維の侵入が観察される。

【0005】また、慢性腎不全とは慢性腎炎症候群の結果、腎機能の劣化により体内の恒常性が維持できなくなった状態である。慢性腎不全の進行を病理学的にみると糸球体硬化と間質線維化の進行である。糸球体硬化症は、メサンギウム領域を中心とした細胞外マトリックスの増生である。メサンギウム硬化症の成分は正常と比較し、著明にIV型コラーゲンなどの糸球体基底膜の成分が増加し、また間質成分であるI型コラーゲンも硬化症部位に一致して増生している。すなわち、慢性に経過する糸球体硬化に対しては、細胞外マトリックスの産生亢進が大きな要因である。ここで慢性腎不全に陥る疾患とは、例えばIgA腎症、糸球体硬化症、膠性増殖性腎炎、糖尿病性腎症、慢性間質性腎炎、慢性糸球体腎炎などを含む。

【0006】また、心筋細胞は高度に分化した細胞で、分裂して増殖する能力を持ち合わせていない。したがって、心臓に何らかの負荷が加わると、心筋細胞はその一つ一つが肥大して取縮力を増大させ、心機能を保とうとする。更に、負荷が長時間持続すると、虚血の要因を中心に多彩な障害が蓄積され、負荷に対する代償機構に破綻をきたし、心筋の取縮力は急激に低下し、心臓のポンプ機能は損なわれて、心不全に陥ることが知られてお

り、心肥大は我が国における心不全の成因として最も大きな部分を占めている。また、心肥大の形成は、心不全発症の最大の危険因子になるばかりでなく、虚血性心疾患や重篤な心室性不整脈の合併率が有意に高くなり、生命予後を独立に規定する要因になっている。心肥大進展時には個々の心筋細胞が著しく肥大するだけでなく、その心筋細胞をしっかりと束ねるために、間質の線維化が促進され、細胞外マトリックスであるコラーゲンが増加する。また、心筋炎・心筋虚血などにより心筋細胞を失うと、コラーゲンが生合成され間隙を置換する。間質の線維化が過剰に進展すると、その結果、心筋は固くなり、弛張が障害される。更に、筋小体機能も低下して心筋の拡張期の弛緩も障害される。その他、術後の瘢痕や熱傷性瘢痕、あるいは強度症、動脈硬化等の細胞外マトリックス産生亢進の病態を示す病気は、何らかの原因によりコラーゲン合成の異常亢進が起り、線維化が進んで組織の硬化変化を生ずることが主要な成因と考えられている。

【0007】また、血管新生においても基底膜及び基底膜中のコラーゲン合成が、重要な役割をはたすことが指摘されている (Maragoudakis, E., Sarmonika, M., and Panoutsacopoulous, M., "J. Pharmacol. Exp. Ther.", 244: 729, 1988; Ingber, D. E., Madri, J. A., and Folkman, J., "Endocrinology", 119: 1768, 1986)。血管新生による疾患としては、例えば、糖尿病性網膜症、後水晶体線維増殖症、角膜移植に伴う血管新生、線内症、眼腫瘍、トロコーマ、幹せん、化膿性肉芽腫、血管瘤、線維性血管腫、肥大型はん瘻、肉芽、リューマチ性関節炎、浮腫性硬化症、アテローム性動脈硬化症、各種腫瘍などが知られている。このようにコラーゲンなどの細胞外マトリックスの産生の亢進の病態を示す病気が大きな問題となっているにもかかわらず、従来では副作用や薬理効果等の種々の面で満足すべき細胞外マトリックス合成抑制剤（例えば、コラーゲン合成抑制剤）は未だ開発されていなかったのである。

【0008】一方、熱ショックタンパク質 (heat shock protein; HSP、ストレスタンパク質ともいう) は、細胞を何らかのストレス、例えば熱、重金属、薬剤、アミノ酸類似体、又は低酸素（低濃度酸素）などで刺激することにより、細胞に発現される一群のタンパク質である。熱ショックタンパク質は、自然界に普遍的に存在しており、細菌、酵母、植物、昆虫、及びヒトを含む高等動物により産生される。HSPは、その種類は多種多様であるが、分子量の大きさからHSP90ファミリー（例えば、90kD又は110kDのHSPなど）、HSP70ファミリー（例えば、70~73kDのHSPなど）、HSP60ファミリー（例えば、57~68kDのHSPなど）、低分子HSPファミリー（例えば、20kD、25~28kD、又は47kDのHSPなど）の4ファミリーに大別することができる。なお、本

明細書においては、特定分子量を有するHSPを、HSPとその直後に記載する数字とによって示すものとし、例えば、分子量47kDのHSPを『HSP47』と称するものとする。以上のように、HSPには多くの種類が存在するが、これらは分子量だけでなく、構造、機能、又は性質などもそれそれ異なるものである。ストレスへの応答に加えて、これらのタンパク質の中には構成的に合成されるものがあり、正常な環境の下で、タンパク質のフォールディング、アンフォールディング、タンパク質サブユニットの会合、タンパク質の膜輸送のような、必須の生理的な役割を演じていることが示されている。熱ショックタンパク質としてのこれらの機能は、分子シャペロンと称される。

【0009】HSP47は、永田等によって1986年に発見されたタンパク質で、分子量47キロダルトンの癌性タンパク質 (pI = 9.0) である。HSP47の発現が増大するにつれて、コラーゲンの合成も増加することが様々な細胞で示されている ("J. Biol. Chem.", 261: 7531, 1986; "Eur. J. Biochem.", 206: 323, 1992; "J. Biol. Chem.", 265: 992, 1990; "J. Clin. Invest.", 93: 2481, 1994)。すなわち、HSP47は、細胞内で小胞体内でのプロコラーゲンのプロセシング、三重鎖ヘリックス形成、あるいは小胞体からゴルジ装置へのプロコラーゲン輸送・分泌という局面で、コラーゲンの特異的分子シャペロンとして機能しているとされているので、増大したHSP47発現は、細胞外マトリックスにおけるコラーゲン分子の蓄積を刺激する。このようにコラーゲン結合熱ショックタンパク質であるHSP47は、発現と同様に機能においても、細胞外マトリックスタンパク質であるコラーゲンに密接に関連した熱ショックタンパク質である。

【0010】

【発明が解決しようとする課題】本発明者らは、上記事情に鑑み、肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥る疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの細胞外マトリックス産生亢進の病態を示す病気の患者の生理学的状態を有効に改善させ、肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥る疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの細胞外マトリックス産生亢進の病態を示す病気を効果的に治療することのできる、細胞外マトリックス合成抑制剤を提供するために、種々検討を重ねてきた。

【0011】上記したように、肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥る疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの線維症は臓器内の細胞外マトリックスの著しく

増加した病態が主病変と理解されている。肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥る疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの細胞外マトリックス産生亢進の病態を示す病気に伴う線維化は、コラーゲン合成増加やコラーゲン分解能の低下により生ずると考えられている。例えば、肝の線維化において、I型、III型、IV型コラーゲンの合成活性化が起こるが、特に主要成分であるI型コラーゲンの合成活性化が重要な意味をもつ。

【0012】こうした状況下で、本発明者らは、意外にも、ニンジン又はコウジン等の成分であるジンセノサイド類、特にジンセノサイドRg₁が、病態を示す組織の細胞におけるHSP47の合成を特異的に抑制することを見出した。すなわち、ジンセノサイド類を投与することにより、細胞内でのHSP47の合成を抑制し、臓器内でのコラーゲン合成を抑制し、ひいては肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥る疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの細胞外マトリックス産生の亢進の病態を示す病気の治療が可能であることを見出したのである。本発明はこうした知見に基づくものであり、肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥る疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの細胞外マトリックス産生の亢進の病態を示す病気を効果的に治療することのできるHSP47合成抑制剤であって、細胞内でのコラーゲンの成熟及び輸送過程に重要な役割を果たしているコラーゲン特異的な分子シャペロンであるHSP47の合成抑制剤を提供することを目的とする。

【0013】

【課題を解決するための手段】従って、本発明は、ジンセノサイド類、特にジンセノサイドRg₁を有効成分として含有することを特徴とする、HSP47合成抑制剤に関する。

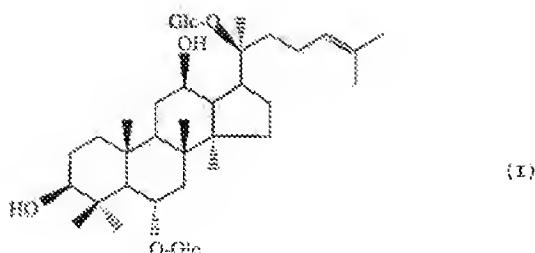
【0014】

【発明の実施の形態】以下、本発明について詳細に説明する。本発明の合成抑制剤は、有効成分としてジンセノサイド類を含有する。本明細書においてジンセノサイド類（ジンセノシド類；ginsenosides）とは、例えば、ジンセノサイドRo（ジンセノシドRo；ginsenoside Ro；チクセツサボニンV；chikusetsusaponin V；サボニンA；saponin A）、ジンセノサイドRa₁（ジンセノシドRa₁；ginsenoside Ra₁）、ジンセノサイドRa₂（ジンセノシドRa₂；ginsenoside Ra₂）、ジンセノサイドRb₁（ジンセノシドRb₁；ginsenoside Rb₁；サ

ボニンD；saponin D）、ジンセノサイドRb₂（ジンセノシドRb₂；ginsenoside Rb₂）、ジンセノサイドRb₃（ジンセノシドRb₃；ginsenoside Rb₃）、ジンセノサイドRc（ジンセノシドRc；ginsenoside Rc）、ジンセノサイドRd（ジンセノシドRd；ginsenoside Rd）、ジンセノサイドRe（ジンセノシドRe；ginsenoside Re）、ジンセノサイドRf（ジンセノシドRf；ginsenoside Rf）、ジンセノサイドRg₁（ジンセノシドRg₁；ginsenoside Rg₁）、ジンセノサイドRg₂（ジンセノシドRg₂；ginsenoside Rg₂；チクセツサボニンI；chikusetsusaponin I）、ジンセノサイドRg₃（ジンセノシドRg₃；ginsenoside Rg₃）、ジンセノサイドRh₁（ジンセノシドRh₁；ginsenoside Rh₁）、ジンセノサイドRh₂（ジンセノシドRh₂；ginsenoside Rh₂）、20-グルコジンセノサイドRf（20-グルコジンセノシドRf；20-glucoginsenoside Rf）、マロニルジンセノサイドRb₁（マロニルギンセノシドRb₁；maronylginsenoside Rb₁）、マロニルジンセノサイドRb₂（マロニルギンセノシドRb₂；maronylginsenoside Rb₂）、マロニルジンセノサイドRc（マロニルギンセノシドRc；maronylginsenoside Rc）、マロニルジンセノサイドRd（マロニルギンセノシドRd；maronylginsenoside Rd）、チクセツサボニンIa（chikusetsusaponin Ia）、チクセツサボニンIb（chikusetsusaponin Ib）、チクセツサボニンIII（chikusetsusaponin III）、チクセツサボニンIV（chikusetsusaponin IV；サボニンB；saponin B）、チクセツサボニンIVa（chikusetsusaponin IVa；サボニンC；saponin C）、プロトバナキサジオール（protopanaxadiol）、プロトバナキサトリオール（protopanaxatriol）、オレアノール酸（oleanolic acid）等、又はこれらの化合物の立体異性体を意味する。本発明においては、それらのジンセノサイド類は、単独で用いることもできるし、あるいは、異なる複数のジンセノサイド類を組み合わせて同時に用いることもできる。

【0015】本発明の合成抑制剤において有効成分として使用することのできるジンセノサイド類としては、特に、ジンセノサイドRg₁が最も好適である。ジンセノサイドRg₁（ジンセノシドRg₁；ginsenoside Rg₁）は、式（I）：

【化1】



(式中、Glcは β -D-グルコピラノシリル基である)で表され、分子式 $C_{42}H_{72}O_{14}$ 及び分子量801、03の化合物であり、例えば、ニンジン又はコウジン等の生薬に含まれている。

【0016】本発明の合成抑制剤に含有されるジンセノサイド類は、化学合成によって、又は天然物から抽出して精製することによって、調製することができる。あるいは、市販品を用いてもよい。本発明の合成抑制剤において有効成分として用いるジンセノサイド類を、天然物から抽出する場合には、例えば、ジンセノサイド類を含有する植物の全体又は一部分（例えば、全草、葉、根、根茎、茎、根皮、若しくは花）をそのまま用いて、又は簡単に加工処理（例えば、乾燥、切断、湯通し、蒸気加熱、若しくは粉末化）したもの（例えば、生薬）を用いて抽出する。抽出条件は一般的に植物抽出に用いられる条件ならば特に制限はない。ジンセノサイド類を含有する植物としては、これに限定するものではないが、例えば、ウコギ科 (Araliaceae) のオタネニンジン (*Panax ginseng* C. A. Meyer; *Panax schinseng* Nees)、トチバニンジン [*Panax japonicus* C. A. Meyer; *Panax schinseng* Nees var. *japonicum* Makino; *Panax pseudo-ginseng* (Willd.) subsp. *japonicus* Harata]、サンシチニンジン [*Panax notoginseng* (Burkitt) F. H. Chen; *Panax sanchi* Hoo; *Panax pseudoginseng* Wallitch var. *notoginseng* (Burkitt) Hoo et Tseng]、又はアメリカニンジン (*Panax quinquefolium* L.) 等を使用することができる。

【0017】本発明におけるジンセノサイド類を生薬から抽出する場合、これに限定するものではないが、例えば、ニンジン又はコウジンから抽出することが好ましい。ニンジン（人参; *Ginseng Radix*）とは、オタネニンジンの細根を除いた根又はこれを軽く湯通ししたものを意味し、それらの部分を単独であるいは任意に組み合わせて使用することができる。また、コウジン（紅参; *Red Ginseng*; *Ginseng Radix Rubra*）とは、オタネニンジンの根

を蒸したものと意味し、それらの部分を単独であるいは任意に組み合わせて使用することができる。

【0018】本発明による合成抑制剤において有効成分として用いることのできるニンジン抽出物又はコウジン抽出物は、前記のジンセノサイド類、特にジンセノサイド Rg₁ を含有していればよく、従って、ニンジン又はコウジンの粗抽出物を用いることができる。本発明で用いることのできるニンジン抽出物又はコウジン抽出物の製造方法としては、ニンジン又はコウジンを、水（例えば、冷水、温水、好ましくは熱湯）によって抽出するか、又は有機溶媒を用いて抽出することによって、得ることができる。有機溶媒としては、例えば、炭素数1～6のアルコール（例えば、メチルアルコール、エチルアルコール、n-ブロピルアルコール、イソブロピルアルコール、若しくはブチルアルコール）、エステル（例えば、酢酸メチル、酢酸エチル、酢酸プロピル、若しくは酢酸ブチル）、ケトン（例えば、アセトン若しくはメチルイソブチルケトン）、エーテル、石油エーテル、n-ヘキサン、シクロヘキサン、トルエン、ベンゼン、炭化水素のハロゲン誘導体（例えば、四塩化炭素、ジクロロメタン、若しくはクロロホルム）、ビリジン、グリコール（例えば、プロピレングリコール、若しくはブチレングリコール）、ポリエチレングリコール、又はアセトニトリルなどを用いることができ、これらの有機溶媒を単独、又は適宜組み合わせ、一定の比率で混合し、更には無水又は含水状態で用いることができる。好ましくは、メチルアルコール等が望ましい。水抽出又は有機溶媒抽出の方法としては、通常の生薬抽出に用いられる方法を用いることができ、例えば、（乾燥）ニンジン又はコウジン1重量部に対し、水又は有機溶媒3～300重量部を用いて、攪拌しながら、その沸点以下の温度で加熱還流、常温で超音波抽出、あるいは冷浸することができる。抽出工程は、通常は5分～7日間、好ましくは10分～24時間実施し、必要に応じて、攪拌等の補助的手段を加えることにより、抽出時間を短縮することができる。

【0019】抽出工程終了後、汎過又は遠心分離等の適当な方法により、水又は有機溶媒抽出液から、不溶物を分離して粗抽出物を得ることができる。なお、本発明の合成抑制剤において、天然物より抽出、分画したジンセノサイド類、特にジンセノサイド Rg₁ を用いる場合には、前記の粗抽出物を特に精製することなく、そのまま使用してもよい。常法による水抽出物又は有機溶媒抽出物の他に、前記の粗抽出物を各種有機溶媒又は吸着剤等により、更に処理した精製抽出物も、本発明の合成抑制剤の有効成分として用いることができる。これらの粗抽出物及び各種の精製処理を終えた精製抽出物を含むニンジン抽出物又はコウジン抽出物は、抽出したままの溶液を用いても、溶媒を濃縮したエキスを用いても良いし、溶媒を留去し乾燥した粉末、更には結晶化して精製した

もの、あるいは粘性のある物質を用いても良く、またそれらの希釈液を用いることもできる。こうして得られたニンジン抽出物又はコウジン抽出物は、ニンジン又はコウジンに含まれるジンセノサイド類を混合物として含み、同時に原料のニンジン又はコウジンに由来する不純物を含んでいる。

【0020】本発明の合成抑制剤は、ジンセノサイド類、又はジンセノサイド類を含有する植物の抽出物、例えば、ジンセノサイド類を含有する生薬の抽出物（特に、ニンジン抽出物又はコウジン抽出物）を、それ単独で、又は好ましくは製剤学的若しくは獣医学的に許容することのできる通常の担体と共に、動物、好ましくは哺乳動物（特にヒト）に投与することができる。投与剤型としては、特に限定がなく、例えば、散剤、細粒剤、顆粒剤、錠剤、カプセル剤、懸濁液、エマルジョン剤、シロップ剤、エキス剤、若しくは丸剤等の経口剤、又は注射剤、外用液剤、軟膏剤、坐剤、局所投与のクリーム、若しくは点眼薬などの非経口剤を挙げることができる。これらの経口剤は、例えば、ゼラチン、アルギン酸ナトリウム、澱粉、コーンスターク、白糖、乳糖、ぶどう糖、マンニット、カルボキシメチルセルロース、デキストリン、ポリビニルピロリドン、結晶セルロース、大豆レシチン、ショ糖、脂肪酸エステル、タルク、ステアリン酸マグネシウム、ポリエチレングリコール、ケイ酸マグネシウム、無水ケイ酸、又は合成ケイ酸アルミニウムなどの賦形剤、結合剤、崩壊剤、界面活性剤、滑沢剤、流動性促進剤、希釈剤、保存剤、着色剤、香料、調味剤、安定化剤、保湿剤、防腐剤、又は酸化防止剤等を用いて、常法に従って製造することができる。例えば、1重量部のジンセノサイドR_{g1}と99重量部の乳糖などを混合して充填したカプセル剤などである。

【0021】非経口投与方法としては、注射（皮下、筋肉内等）、又は直腸投与等が例示される。これらのなかで、注射剤が最も好適に用いられる。例えば、注射剤の調製においては、有効成分としてのジンセノサイド類（特にジンセノサイドR_{g1}）、又はジンセノサイド類を含有する植物の抽出物、例えば、ジンセノサイド類を含有する生薬の抽出物（特に、ニンジン抽出物又はコウジン抽出物）の他に、例えば、生理食塩水若しくはリンゲル液等の水溶性溶剤、植物油若しくは脂肪酸エステル等の非水溶性溶剤、ブドウ糖若しくは塩化ナトリウム等の等張化剤、溶解補助剤、安定化剤、防腐剤、懸濁化剤、又は乳化剤等を任意に用いることができる。また、本発明の合成抑制剤は、徐放性ポリマーなどを用いた徐放性製剤の手法を用いて投与してもよい。例えば、本発明の合成抑制剤をエチレンビニル酢酸ポリマーのペレットに取り込ませて、このペレットを治療すべき組織中に外科的に移植することができる。

【0022】本発明の合成抑制剤は、これに限定されるものではないが、ジンセノサイド類を、0.01～99

重量%、好ましくは0.1～80重量%の量で含有することができる。また、ジンセノサイド類を含有する植物の抽出物、例えば、ジンセノサイド類を含有する生薬の抽出物（特に、ニンジン抽出物又はコウジン抽出物）を有効成分として含有する本発明の合成抑制剤は、その中に含まれるジンセノサイド類（特にジンセノサイドR_{g1}）が前記の量範囲になるように適宜調整して、調製することができる。なお、ジンセノサイド類を含有する植物の抽出物、例えば、ジンセノサイド類を含有する生薬の抽出物（特に、ニンジン抽出物又はコウジン抽出物）を有効成分として含有する合成抑制剤を、経口投与用製剤とする場合には、製剤学的に許容することのできる担体を用いて、製剤化することが好ましい。

【0023】本発明の合成抑制剤を用いる場合の投与量は、病気の種類、患者の年齢、性別、体重、症状の程度、又は投与方法などにより異なり、特に制限はないが、ジンセノサイドR_{g1}量として通常成人1人当たり1mg～1.0g程度を、1日1～4回程度にわけて、経口的に又は非経口的に投与する。更に、用途も医薬品に限定されるものではなく、種々の用途、例えば、機能性食品や健康食品として飲食物の形で与えることも可能である。

【0024】

【作用】上記したように、本発明の合成抑制剤に含有されるジンセノサイド類、特にジンセノサイドR_{g1}は、細胞内のHSP47合成を特異的に抑制する作用があるので、前記ジンセノサイド類を投与すると細胞内でのHSP47合成が特異的に減少し、コラーゲンの生合成が抑制される。その結果、細胞外マトリックス産生も抑制されることになる。従って、前記ジンセノサイド類は、コラーゲンの増加を伴う細胞外マトリックス産生亢進の病態を示す病気、例えば肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥る病態）、心肥大、術後の瘢痕や致傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの予防及び治療に使用することができる。すなわち、本発明の合成抑制剤は、コラーゲン特異的シャペロンであるHSP47の合成を抑制することによりコラーゲンの合成を抑制する。

【0025】また、前記のように、血管新生においても、基底膜及び基底膜中のコラーゲン合成が重要な役割をはたすことが指摘されているので、本発明の合成抑制剤は、血管新生の異常増殖に基づく多くの疾患の予防治療薬として極めて有用であり、先に述べたような各疾患、すなわち糖尿病性網膜症、後水晶体線維増殖症、角膜移植に伴う血管新生、緑内症、眼瞼癌、トラコーマ、乾せん、化膿性肉芽腫、血管腫、線維性血管腫、肥大型はん瘡、肉芽、リューマチ性関節炎、浮腫性硬化症、アテローム性動脈硬化症及び各種腫瘍などに用いることができる。更に、I型コラーゲンとフィブロネクチンを基

本骨格とする間質 (interstitial stroma) が癌の転移において、離脱した癌細胞が近傍の脈管に侵入するまでのガイド役を果たすことが、明らかとなっているので ("BIO THERAPY", 7(8): 1181, 1993)、本発明の合成抑制剤を投与することにより、癌の転移を抑制することも可能である。

【0026】

【実施例】以下、実施例によって本発明を具体的に説明するが、これらは本発明の範囲を限定するものではない。

実施例1：抗HSP47ポリクローナル抗体の作製

(1) 抗HSP47ポリクローナル抗体の調製

ヒトHSP47のN末端から2～16番目のアミノ酸配列に対応するアミノ酸15個からなるペプチド（以下、ヒトHSP47ペプチド（2～16）と称する）を自動ペプチド合成装置（PSSM-Sシステム、島津製作所）を用いて作製し、スクシニミジル4-(p-マレイミドフェニル)ブチレート（SMPB；succinimidyl 4-(p-maleimidophenyl)butyrate）を架橋剤として用い、常法 ("Biochemistry", 18: 690, 1979)によりラクトグロブリンと結合させ、感作抗原を作製した。この感作抗原150μgを含むリン酸緩衝生理食塩水〔組成：KC1=0.2g/1, KH₂PO₄=0.2g/1, NaCl=8g/1, Na₂HPO₄（無水）=1.15g/1；以下PBS（-）と称する；コスモバイオ、カタログ番号320-01〕0.2mlと、等量のフロイント完全アジュvant（ヤトロン、カタログ番号RM606-1）とを混和し、得られた混合液0.2mlを、ルーラット（6週齢、雌性；日本クレア）の皮下に投与し、免疫した。同様の方法で第2次及び第3次免疫を繰り返した後、アジュvant（Hunter's TiterMax；CytRx Corporation、米国ジョージア州）を用いて6回免疫感作を行った。感作動物より採血し、常法により血清を分離して採取し、以下に示す酵素抗体法（ELISA法）及びウェスタンプロット法によって血清中の抗体価を測定した。

【0027】(2) 酵素抗体法（ELISA法）による抗HSP47ポリクローナル抗体特性の評価

前項（1）で調製したヒトHSP47ペプチド（2～16）をPBS（-）に溶解し、10μg/mlの濃度のペプチド溶液を調製し、リジットアセイプレート（ファルコン、カタログ番号3910）の各ウェルに前記ペプチド溶液を50μlずつ滴下した。最も外側のウェルにはPBS（-）50μlのみを入れ、湿潤下で4℃にて一晩放置した後、前記ペプチド溶液を捨て、PBS（-）を用いて各ウェルを洗浄した後、1%ウシ血清アルブミン（以下、BSAと略称する）を含むPBS（-）100μlを各ウェルに入れ、室温下で1時間放置した。PBS（-）で3回洗浄した後、前項（1）で取得したルーラット血清50μlを各ウェルに入れ、1時間室温にて放置した。PBS（-）で3回洗浄した後、各ウェルに

2次抗体としてベルオキシダーゼ標識抗ラットIgG 50μlを入れ、室温下で1時間放置した。PBS（-）で3回洗浄した後、過酸化水素水4μlを加えた0.1Mクエン酸バッファー（pH4.5）10mlにオーフェニレンジアミン（OPD）クプレット（シグマ、カタログ番号P8287）1個（10mg）を溶解して調製した基質液100μlずつを各ウェルに滴下し、室温にて遮光下で30分間放置した後、各ウェルの492nmの吸光度をマイクロプレートリーダー（東ソー、MPR-A41型）にて測定した。抗体価の上昇が確認された血清を抗ヒトHSP47ポリクローナル抗体として以下の実施例に用いた。

【0028】(3) ウェスタンプロット法による抗HSP47ポリクローナル抗体特性の評価

Lacemm1のバッファー系（Laemmli, N. K., "Nature", 283: pp. 249-256, 1970)を用いて、HeLa細胞のライセートのドデシル硫酸ナトリウム（SDS）ポリアクリルアミドゲル電気泳動を、以下の方法に従って行った。濃縮ゲルの調製は次のように行った。蒸留水6.1ml、0.5Mトリス（バイオ・ラッド、カタログ番号161-0716）-HCl（pH6.8）2.5ml、10%SDS（バイオ・ラッド、カタログ番号161-0301）1.100μl、及び30%アクリルアミド（バイオ・ラッド、カタログ番号161-0101）/N,N'-メチレンビスアクリルアミド（バイオ・ラッド、カタログ番号161-0201）1.3mlを混合して、15分間脱気し、10%過硫酸アンモニウム（バイオ・ラッド、カタログ番号161-0700）50μl及びN,N,N',N'-テトラメチルエチレンジアミン（以下、TEMEDと略称する）（バイオ・ラッド、カタログ番号161-0800）10μlを加えて、濃縮ゲルを調製した。また、分離ゲルの調製は次のように行った。蒸留水4.045ml、1.5Mトリス-HCl（pH8.8）2.5ml、10%SDS 1.00μl、及び30%アクリルアミド/N,N'-メチレンビスアクリルアミド3.3mlをゆっくり混合して、15分間アスピレーターで脱気し、10%過硫酸アンモニウム50μl、及びTEMED5μlを加えた。泳動バッファーとしては、トリス9.0g、グリシン（バイオ・ラッド、カタログ番号161-0717）43.2g、及びSDS3.0gに蒸留水を加えて600mlにし、これを蒸留水で5倍希釈したものを用いた。サンブルバッファーは、蒸留水2ml、2Mトリス-HCl（pH6.8）500μl、SDS0.32g、β-メルカプトエタノール800μl、及び0.05%（w/v）ブロモフェノールブルー（バイオ・ラッド、カタログ番号161-0404）400μlを混合したもの用いた。

【0029】5%二酸化炭素条件下で、37℃で、10%非懸衡ウシ胎児血清（以下、FBSと略称する）を含むMEM培地中でHeLa細胞を培養し、そのライセートを調製した。得られたHeLa細胞ライセートのSD

S-ポリアクリルアミドゲル電気泳動を行った後、 $0.4\text{ }\mu\text{m}$ ニトロセルロース膜 (Schleicher & Schuell, カタログ番号401196) にゲルを密着させ、タンパク質転写装置 (Trans-Blot Electrophoretic Transfer Cell : バイオ・ラッド) を用いて、室温にて100Vで、3時間ブロッティングを行った。プロッティングバッファーとしては 0.025M トリス及び 0.192M グリシンよりなり pH 8.5に調整されたトリスグリシンバッファー (Tris Gly Running and Blotting Buffer ; Baprotech, 米国マサチューセッツ州, カタログ番号5A100034) にメチルアルコールを20%になるように加えて調製したバッファーを用いた。プロッティング後、5%スキムミルク (雪印乳業) を含むPBS (-) 溶液にニトロセルロース膜を室温にて30分間浸し、ブロッキングを行った。プロッキング後、スクリーナーブロッター (サンプラテック) を用いて、前項(1)で取得したルーラット血清を1次抗体として、1次抗体反応を行った。1次抗体反応は、2%スキムミルク (雪印乳業) を含むPBS (-) にて10倍希釈した前記ルーラット血清 $200\text{ }\mu\text{l}$ で、室温にて120分間行った。1次抗体反応終了後、スロー・ロッキング・シェイカーを用いて、PBS (-) で5分間の振盪を2回、 0.1% Tween 20 (バイオ・ラッド, カタログ番号170-6531) を含むPBS (-) 溶液で15分間の振盪を4回、更にPBS (-) で5分間の振盪を2回行うことにより、ニトロセルロース膜を洗浄した。洗浄終了後、ベルオキシダーゼ標識ヤギ抗ラットIgG抗体 (Southern Biotechnology, カタログ番号3030-05) を、2%スキムミルクを含むPBS (-) 溶液で5000倍に希釈した溶液 5 ml を用いて、2次抗体反応を2時間行った。反応終了後、PBS (-) 溶液、及び 0.1% Tween 20を含むPBS (-) 溶液で、1次抗体反応後の洗浄と同じ条件下にてニトロセルロース膜の洗浄を行った。

【0030】余分なPBS (-) 溶液を除去した後、ウェスタンブロッティング検出試薬 (ECL Western blotting detection reagent ; アマーシャム, カタログ番号RP N2106) をニトロセルロース膜上に振りかけ、1分間室温にて静置した後、余分な検出試薬を除去し、ニトロセルロース膜をラップに包み、反応面をX線フィルム (コダック X-DMAT, AR, カタログ番号165-1454) に密着させて露光させた。現像後、HSP 4.7に相当する分子量4.7キログルトン付近のバンドを測定することによって、抗HSP 4.7ポリクローナル抗体の反応性の検討を行った。抗体価の上昇が確認された血清を、抗ヒトHSP 4.7ポリクローナル抗体として、以下の実施例に用いた。

【0031】実施例2：ヒト培養癌細胞のHSP発現量の測定

(1) ヒト培養癌細胞の培養

ヒト乳癌細胞株MCF 7 (ATCC HTB 22) を、 10^{-8}M β-エストラジオール及び10%非懸濁化P

B Sを含むRPMI 1640培地中で、5%二酸化炭素条件下で、熱ショック処理時以外は、37°Cで培養した。

【0032】(2) ジンセノサイドRg₁処理及び熱ショック処理

播種2日後の前記ヒト乳癌細胞株MCF 7の培地中に、最終濃度 $100\text{ }\mu\text{M}$ になるよう前記式(1)で表されるジンセノサイドRg₁ (松浦薬業) を添加し、24時間培養した。その後、45°Cにて15分間熱ショック処理をしてから、37°Cにて終夜培養した。対照試験は、ジンセノサイドRg₁を添加しないこと以外は前記と同様に実施した。

【0033】(3) ヒト培養癌細胞でのHSP発現量の測定

前項(2)で処理した各細胞を、以下に示す方法によりホモジナイズし、HSP発現量をウェスタンプロット法にて測定した。すなわち、前項(2)で処理した細胞をPBS (-) で洗浄した後、ライシスバッファー (lysis buffer) (1.0% NP-40, 0.1% 胰島化ナトリウム、 5.0 mM トリス-HCl (pH 8.0)、 5 mM -EDTA、 2 mM -N-エチルマレイミド、 2 mM フェニルメチルスルホニルフルオリド、 $2\text{ }\mu\text{g}/\text{ml}$ ロイペプチド及び $2\text{ }\mu\text{g}/\text{ml}$ ペプスタチン) 1 ml を加え、氷上で20分間静置した。その後、4°Cで12000 rpmにて、20分間、遠心を行った。遠心後の上清 $10\text{ }\mu\text{l}$ をPBS (-) $790\text{ }\mu\text{l}$ に加え、更にプロテインアッセイ染色液 (Dye Reagent Concentrate ; バイオラッド, カタログ番号500-0006) $200\text{ }\mu\text{l}$ を加えた。5分間、室温にて静置した後、 595 nm で吸光度を測定してタンパク質定量を行った。タンパク質定量を行った試料を用いて、 $1\text{ }\mu\text{g}/\text{ml}$ のバッファー系にて、等量のタンパク質を含むライセートのSDSポリアクリルアミドゲル電気泳動を行った。電気泳動後、実施例1で述べた方法に従って、プロッティング及びそれに続くブロッキングを行った。すなわち、タンパク質転写装置 (Trans-Blot Electrophoretic Transfer Cell : バイオ・ラッド) を用いて、室温にて100Vにて、 $0.45\text{ }\mu\text{m}$ ニトロセルロース膜 (Schleicher & Schuell, カタログ番号401196) にゲルを密着させ、3時間プロッティングを行った。プロッティングバッファーとしては、前記実施例1(3)で用いたバッファーと同じものを用いた。プロッティング後、ニトロセルロース膜を10%スキムミルク (雪印乳業)-PBS (-) 溶液に室温にて30分間、インキュベートし非特異的結合をブロックした。

【0034】ブロッキング後、ニトロセルロース膜の上で、実施例1で製造した抗ヒトHSP 4.7ラットポリクローナル抗体により、1次抗体反応を行った。その後、PBS (-) で5分間ずつ、溶液を取り替えて2回の洗浄をスロー・ロッキング・シェイカーによって行い、更

にPBS (-) - 0.1%Tween 20 (バイオ・ラット、カタログ番号170-6531) 溶液で15分間ずつ、溶液を取り替えて4回の洗浄を行った。最終的に、PBS (-) で5分間ずつ、2回の洗浄を行った。洗浄終了後、ペルオキシダーゼ標識ヤギ抗ラット IgG 抗体 (Southern Biotechnology, カタログ番号3030-05) を、2%スキムミルクを含むPBS (-) 溶液で5000倍に希釈して調製した抗体溶液5mlを用いて、2時間、2次抗体反応を行った。反応終了後、ニトロセルロース膜に関して、PBS (-) 溶液で5分間ずつ溶液を変えて2回、更にPBS (-) - 0.1%Tween 20 溶液で15分間ずつ溶液を変えて5回の洗浄をスロー・ロッキング・シェイカーにより行った。最後にPBS (-) 溶液で5分間ずつ2回の洗浄を行った。余分なPBS (-) 溶液を除去した後、ウェスタンブロッティング検出試薬 (ECL Westernblotting detection reagent : Amersham, カタログ番号RPN2106) をニトロセルロース膜上に振りかけ、1分間インキュベートした後、余分な検出試薬を除去し、ニトロセルロース膜をラップに包み、反応面をX線フィルム (コダック X-Omat, 級, カタログ番号165-1454) に密着させて露光し、現像してHSP47の有無の検討を行った。

【0035】対照試験、すなわち、ジンセノサイドRg₁を添加しなかった乳癌細胞株MCF7では、分子量約47kDのバンドが一本検出された。ジンセノサイドRg₁

を添加した乳癌細胞株MCF7では、相当するバンドが極めて薄いバンドであった。すなわち、ジンセノサイドRg₁は、HSP47の発現を抑制する合成抑制剤の活性を有するものと結論することができ、この事実は、ジンセノサイドRg₁が細胞外マトリックス産生の亢進に抑制的に働くことを示している。

【0036】

【発明の効果】以上詳述したように、本発明の合成抑制剤は、例えば、肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥る疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの細胞外マトリックス産生の亢進の病態を示す病気に罹患した細胞にみられるコラーゲン合成亢進を改善する作用を有する。従って、本発明による合成抑制剤を投与することにより、臓器、組織の線維化、硬化が阻止され、その結果、前記病気の患者の生理学的状態を有効に改善させ、前記病気を効果的に治療することができる。また、本発明の合成抑制剤は、血管新生の異常増殖を伴う各種疾患の予防治療にも有用である。更に、I型コラーゲンとフィブロネクチンを基本骨格とする間質が、癌の転移において離脱した癌細胞が近傍の脈管に侵入するまでのガイド役を果たすことが、明らかとなっているので、本発明の合成抑制剤を投与することにより、癌の転移を抑制することも可能である。

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DETAILED DESCRIPTION

[Detailed Description of the Invention]**[0001]**

[Field of the Invention] The molecular weight in which this invention contains JINSENO sides, especially JINSENO side Rg₁, as an active principle is related with synthetic inhibitor of the heat shock protein (HSP47 is called hereafter) of 47 kilodalton (kD). Especially HSP47 synthetic inhibitor of this invention by controlling composition of collagen in an organ Liver cirrhosis, An interstitial lung disease, chronic renal failure (or chronic renal failure ***** disease), cardiac hypertrophy, The keloid and the hypertrophic scar which are produced after a postoperative scar, a burn nature scar, a traffic accident, etc., The physiological status of the sick patient who shows the symptoms of extra-cellular-matrix (extracellular matrix) production sthenia of a scleroderma, arteriosclerosis, or articular rheumatism is made to improve effectively, Liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ***** disease), The illness which shows the symptoms of extracellular-matrix-production sthenia of the keloid produced after cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc., a hypertrophic scar, a scleroderma, arteriosclerosis, or articular rheumatism can be treated effectively.

[0002]

[Description of the Prior Art] In recent years, the illness which shows the symptoms of sthenia of production of extra-cellular matrices, such as collagen, poses a big problem. With the illness which shows the symptoms of sthenia of extracellular matrix production here. For example, keloid, a hypertrophic scar, a scleroderma, arteriosclerosis, or articular rheumatism produced after liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ***** disease), cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc. is included.

[0003] For example, the liver cirrhosis said for the deceased to climb at least our country also in

about 20,000 people per year is the hepatic fibrosis of ***** covering the flume crack which is a sick general term which becomes hard since liver is growth of connective tissue, and is an end image of various chronic liver disease, and the whole liver. That is, in the chronic hepatitis which attains to a long period of time, liver injury, such as inflammation, carries out fibrosis of the liver with remarkable sthenia of extra-cellular-matrix (especially I-beam collagen) production of fibroblast, Ito cells, etc. If the fibrosis of a liver advances chronically, increasingly normal liver regeneration is blocked, hepatocytes are replaced, and the extra-cellular matrix which makes I-beam collagen fibroblast with a subject will occupy most portion of a hepatic tissue, and will result in the liver cirrhosis which consists of many *****. The abnormalities of the blood flow which a fiber septum progresses to the whole liver and produces as a result with advance of liver cirrhosis, It also becomes a cause which pushes the denaturation of a hepatocyte further, and the vicious circle in liver cirrhosis will continue, and also a lot of colloid fibers in liver are generated by various causes, such as alcohol, a virus, and autoimmunity, a necrosis and afunction of hepatocytes arise by them, and a cirrhotic patient dies finally. Although I-beam collagen occupies about 2% of a total protein amount in a normal liver, if it serves as liver cirrhosis, it will come to occupy 10 to 30%.

[0004]An interstitial lung disease is a disease group characterized by the chronic inflammation (alveolitis alveolitis) of an alveolus and not only an alveolar duct but the lower respiratory tract which also often involves in respiratory bronchiole and an end bronchial tube, the fibrosis of the stromata which are the result, and alveolar endogenous-fibers-ization. With an interstitial lung disease here, diffusion interstitial lung diseases, such as pneumonitis and fibroid lung, idiopathic pulmonary fibrosis, the penetrable pulmonary edema, a collagen disease of lung, sarcoidosis, etc. are included, for example. In an interstitial lung disease, superfluous production and accumulation of the extra-cellular matrix are accepted in the fibrosis organization. That is, in the lung fibrosis organization of an interstitial lung disease, accumulation of a Tsuguaki I-beam and III type collagen is seen by enlarged stromata, the alveolar septa which carried out thickening to the early stage of fibrosis are piled up, a stadium reaches an advanced stage, I-beam collagen increases in the second half, and especially III type collagen turns into main collagen. Basement membrane is destroyed at an early stage, and invasion of the collagen fiber by the side of an alveolar space is observed.

[0005]Chronic renal failure is in the state where it became impossible to maintain homeostasis in the living body by desolation of a kidney function, as a result of chronic nephritic syndrome. When advance of chronic renal failure is seen pathologically, it is advance of glomerulosclerosis and stromata fibrosis. The glomerulosclerosis is the hyperplasia of the extra-cellular matrix centering on a mesangium field. It compares that it is normal, and the ingredient of glomerular basement membrane, such as IV type collagen, increases to Tsuguaki, and the ingredient of mesangium sclerosis is carrying out hyperplasia also of the I-

beam collagen which is a stromata ingredient in accordance with the sclerosis part. That is, it is a factor with major production sthenia of an extra-cellular matrix to the glomerulosclerosis which passes in chronicity. With a ***** disease, IgA glomerulonephritis, focal glomerular sclerosis, membranoproliferative glomerulonephritis, diabetic nephropathy, a chronic interstitial nephritis, chronic glomerulonephritis, etc. are included in chronic renal failure here, for example.

[0006]A cardiac muscle cell is a cell which specialized highly, and does not have the capability with it to divide and increase. Therefore, if a certain load is added to the heart, the each will get fat, and a cardiac muscle cell will increase a shrinkage force, and will try to maintain cardiac performance. If load continues for a long time, a variegated obstacle will be accumulated focusing on the factor of the ischemia, cause a breakdown to the compensatory mechanism over load, the shrinkage force of a myocardium declines rapidly, and the pumping ability of the heart is spoiled -- cardiac insufficiency -- ***** -- things are known and cardiac hypertrophy occupies the biggest portion as the origin of the cardiac insufficiency in our country. The merger rate of ischemic heart disease or critical ventricular arrhythmia becomes high intentionally, and formation of cardiac hypertrophy not only becomes the greatest risk factor of the onset of cardiac insufficiency, but has become a factor which specifies a vital prognosis independently. In order that each cardiac muscle cell not only gets fat remarkably, but may bundle the cardiac muscle cell firmly at the time of cardiac hypertrophy progress, the fibrosis of stromata is promoted and collagen which is an extra-cellular matrix increases. If a cardiac muscle cell is lost by myocarditis, myocardial ischemia, etc., collagen will be biosynthesized and a gap will be replaced. If the fibrosis of stromata progresses superfluously as a result, a myocardium will become hard and the obstacle of the extension will be carried out. A muscle snug function also falls and the obstacle also of the relaxation of the diastole of a myocardium is carried out. In addition, the gastric upset of collagen synthesis happens by a certain cause, and the illnesses which show the symptoms of extracellular-matrix-production sthenia of a postoperative scar, a burn nature scar or a scleroderma, arteriosclerosis, etc. are considered to be the origins with main fibrosis progressing and producing hardening change of an organization.

[0007]Also in the vascularization, the collagen synthesis in basement membrane and basement membrane, An important role. Achieving is pointed out (). [Maragoudakis and] E., Sarmonika, M., and and. Panoutsacopoulous and M., "J. Pharmacol. Exp. Ther.", 244 : 729, 1988 ; Ingber, D. E., Madri, J. A., and Folkman, J., "Endocrinology", 119: 1768, 1986. As a disease by the vascularization, for example Diabetic retinopathy, back fibrae-lentis vegetation, The vascularization accompanying a corneal transplantation, glaucoma, an eye tumor, trachoma, a trunk plug, the pyogenic granuloma, hemangioma, angioma fibrosum, a tumescent scar, granulation, rheumatism nature arthritis, the scleredema, atherosclerosis,

various tumors, etc. are known. Thus, although the illness which shows the symptoms of sthenia of production of extra-cellular matrices, such as collagen, poses a big problem, In the former, extra-cellular-matrix synthetic inhibitor (for example, collagen synthesis depressant) should be satisfied with various fields, such as side effects and a medicinal value, of synthetic inhibitor was not yet developed.

[0008]On the other hand, a heat shock protein (it is also called heat shock protein;HSP and stress protein), a group revealed by the cell by stimulating a cell by a certain stress, for example, heat, a heavy metal, drugs, an amino acid analog, or hypoxia (low concentration oxygen) -- it is protein. The heat shock protein exists in the nature universally.

It is produced by bacteria, yeast, vegetation, an insect, and the higher animal including Homo sapiens.

Although the kind is various, HSP, From the size of a molecular weight to HSP90 family (for example, HSP of 90kD or 110kD, etc.). It can divide roughly into 4 of HSP70 family, HSP(s)(for example, HSP of 70 - 73kD, etc.)60 family, and low molecule HSP(s) (for example, HSP of 57 - 68kD, etc.) families (for example, HSP of 20kD, 25 - 28kD, or 47kD, etc.) families. In this Description, the number indicated to be HSP immediately after that shall show HSP which has specific molecular weight, for example, HSP of molecular weight 47kD shall be called "HSP47." As mentioned above, although many kinds exist in HSP, these differ not only in a molecular weight but in structure, a function, or character etc., respectively. the response to stress -- in addition, some of these protein being compounded compositionally and under normal environment, A thing like a meeting of proteinic folding, unfolding, and a protein subunit and proteinic membrane transport for which the indispensable physiological role is played is shown. These functions as a heat shock protein are called a molecular chaperone.

[0009]HSP47 is the protein discovered by Hitoshi Nagata in 1986, and is basic protein ($pI=9.0$) with a molecular weight of 47 kilodalton. As the manifestation of HSP47 increases, It is shown by various cells that composition of collagen also increases ("J. Biol. Chem." 261: 7531 and 1986;" Eur. J. Biochem." 206:323 and 1992;" J.). Biol. Chem.", 265 : 992, 1990 ; "J. Clin. Invest.", 94:2481, 1994. Namely, HSP47 is an aspect of affairs of processing of the procollagen within an endoplasmic reticulum, 3 heavy-chain helix formation, or the procollagen transportation and secretion to Golgi apparatus from an endoplasmic reticulum in intracellular. Since it is functioning as a specific molecular chaperone of collagen, the HSP47 manifestation which increased stimulates accumulation of the collagen molecule in an extra-cellular matrix. Thus, HSP47 which is a collagen binding heat shock protein is the heat shock protein which related to collagen which is extracellular matrix protein closely also in the function like the manifestation.

[0010]

[Problem to be solved by the invention] This invention persons in view of the above-mentioned

situation Liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ***** disease), The keloid and the hypertrophic scar which are produced after cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc., The physiological status of the sick patient who shows the symptoms of extracellular-matrix-production sthenia of a scleroderma, arteriosclerosis, or articular rheumatism is made to improve effectively, Liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ***** disease), The keloid and the hypertrophic scar which are produced after cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc., In order to provide the extra-cellular-matrix synthetic inhibitor which can treat effectively the illness which shows the symptoms of extracellular-matrix-production sthenia of a scleroderma, arteriosclerosis, or articular rheumatism, examination was come in piles variously.

[0011]As described above, liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ***** disease), The symptoms which the extra-cellular matrix in an organ is remarkable, and fibrosing diseases produced after cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc., such as keloid, a hypertrophic scar, a scleroderma, arteriosclerosis, or articular rheumatism, increased are understood to be the main lesion. Liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ***** disease), It is thought that the fibrosis which shows the symptoms of extracellular-matrix-production sthenia of the keloid produced after cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc., a hypertrophic scar, a scleroderma, arteriosclerosis, or articular rheumatism and by which it is accompanied sick is produced due to the increase in collagen biosynthesis or the fall of collagen degradation ability. For example, in the fibrosis of a liver, although synthesis-activities-ization of an I-beam, an III type, and IV type collagen takes place, synthesis-activities-ization of I-beam collagen which is especially a basic component has an important meaning.

[0012]Under such a situation, this invention persons found out that the JINSENO sides which are ingredients, such as a ginseng or Ginseng Radix Rubra, also unexpectedly, especially JINSENO side Rg₁, controlled specifically composition of HSP47 in the cell of the organization which shows symptoms. Namely, composition of intracellular HSP47 is controlled by prescribing JINSENO sides for the patient, Control the collagen synthesis within an organ and As a result, liver cirrhosis, an interstitial lung disease, It found out that the sick therapy which shows the symptoms of extracellular-matrix-production sthenia of the keloid produced after chronic renal failure (or chronic renal failure ***** disease), cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc., a hypertrophic scar, a scleroderma, arteriosclerosis, or articular rheumatism was possible. This invention is based on such knowledge and Liver cirrhosis, an interstitial lung disease, Chronic renal failure (or chronic renal failure ***** disease), cardiac hypertrophy, a postoperative scar and burn nature

scar, The keloid, the hypertrophic scar, the scleroderma, arteriosclerosis which are produced after a traffic accident etc., Or it is HSP47 synthetic inhibitor which can treat effectively the illness which shows the symptoms of sthenia of extracellular matrix production, such as articular rheumatism, the collagen which has played the role important for maturation and the transport process of intracellular collagen -- it aims at providing synthetic inhibitor of HSP47 which is a specific molecular chaperone.

[0013]

[Means for solving problem]Therefore, this invention relates to HSP47 synthetic inhibitor containing JINSENO sides, especially JINSENO side Rg₁ as an active principle.

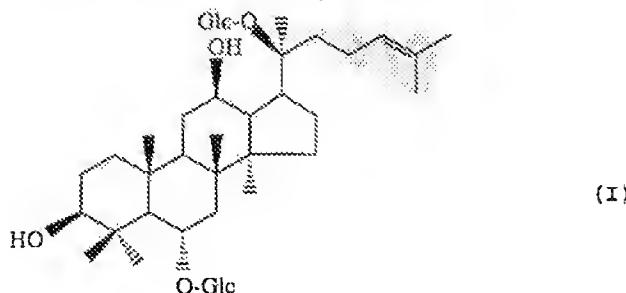
[0014]

[Mode for carrying out the invention]Hereafter, this invention is explained in detail. Synthetic inhibitor of this invention contains JINSENO sides as an active principle. In this Description, with JINSENO sides (GINSENOSHIDO; ginsenosides). For example, the JINSENO side Ro (GINSENOSHIDO Ro;ginsenoside Ro; chikusetsu-saponin V;chikusetsusaponinV; saponin A;saponin A), JINSENO side Ra₁ (GINSENOSHIDORa₁;ginsenoside Ra₁), JINSENO side Ra₂ (GINSENOSHIDORa₂;ginsenoside Ra₂), JINSENO side Rb₁ (GINSENOSHIDORb₁;ginsenoside Rb₁; saponin D;saponin D), JINSENO side Rb₂ (GINSENOSHIDORb₂;ginsenoside Rb₂), JINSENO side Rb₃ (GINSENOSHIDORb₃;ginsenoside Rb₃), The JINSENO side Rc (GINSENOSHIDO Rc;ginsenoside Rc), The JINSENO side Rd (GINSENOSHIDO Rd;ginsenoside Rd), JINSENO side Re (GINSENOSHIDO Re;ginsenoside Re), JINSENO side Rf (GINSENOSHIDO Rf;ginsenosideRf). JINSENO side Rg₁ (GINSENOSHIDORg₁;ginsenoside Rg₁), JINSENO side Rg₂ (GINSENOSHIDORg₂;ginsenoside Rg₂; chikusetsu-saponin I;chikusetsusaponinI), JINSENO side Rg₃ (GINSENOSHIDORg₃;ginsenosideRg₃), JINSENO side Rh₁ (GINSENOSHIDORh₁;ginsenoside Rh₁), JINSENO side Rh₂ (GINSENOSHIDORh₂;ginsenoside Rh₂), 20-guru KOJINSENO side Rf (20-guru KOGINSENOSHIDO Rf;20-glucoginsenoside Rf), Malonyl JINSENO side Rb₁ (malonyl GINSENOSHIDORb₁;maronylginsenoside Rb₁), Malonyl JINSENO side Rb₂ (malonyl GINSENOSHIDORb₂;maronylginsenoside Rb₂), the malonyl JINSENO side Rc (malonyl GINSENOSHIDORc;maronylginsenosideRc), The malonyl JINSENO side Rd (malonyl GINSENOSHIDO Rd;maronylginsenoside Rd), The chikusetsu-saponin Ia (chikusetsusaponin Ia), The chikusetsu-saponin Ib (chikusetsusaponin Ib), The chikusetsu-saponin III (chikusetsusaponin III), The chikusetsu-saponin IV (chikusetsusaponinIV; saponin B;saponin

B), The chikusetsu-saponin IVa (chikusetsusaponinIVa; saponin C;saponin C), Protopanaxadiol (protopanaxadiol), pro TOPANAKISA triol (protopanaxatriol), oleanolic acid (oleanolic acid), etc. mean the stereoisomeric form of these compounds. In this invention, those JINSENO sides can also be simultaneously used combining several JINSENO sides which can also use independently or are different.

[0015]Especially as JINSENO sides which can be used as an active principle in synthetic inhibitor of this invention, JINSENO side Rg₁ is the most preferred. JINSENO side Rg₁

(GINSENOSHIDORg₁;ginsenoside Rg₁) is formula (I). : [Chemical formula 1]



It is expressed with (the inside of a formula and Glc are beta-D-glucopyranosyl groups), and it is a compound of molecular formula C₄₂H₇₂O₁₄ and the molecular weight 801.03, for example,

is contained in crude drugs, such as a ginseng or Ginseng Radix Rubra.

[0016]The JINSENO sides contained in synthetic inhibitor of this invention can be prepared chemosynthesis or by extracting from a natural product and refining. Or a commercial item may be used. In extracting the JINSENO sides used as an active principle in synthetic inhibitor of this invention from a natural product, For example, it extracts using what (for example, crude drug) carried out processing treatment (for example, desiccation, cutting, parboiling, steam heating, or disintegration) simply using vegetable [containing JINSENO sides / the whole or some (for example, the entire plant, a leaf, a root a rhizome a stem a cortex or a flower) of] as it is. If extraction conditions are conditions generally used for vegetable extraction, there will be no restriction in particular. As vegetation containing JINSENO sides, although it does not limit to this, they are araliaceous (Araliaceae) Panax schinseng (Panax ginseng C.A.Meyer;Panax schinseng Nees) and Panax japonicus, for example. [Panaxjaponicus C.A.Meyer;Panax schinsengNees var.japonicum Makino;Panax pseudo-ginseng (Will.) subsp. japonicus Hara] SANSHI thynnine gin [Panax notoginseng (Burkhill) F.H.Chen;Panax sanchi Hoo;Panaxpseudo-ginseng Wallich var.notoginseng (Burkhill) Hoo et Tseng] Or a U.S. ginseng (Panax quinquefolium L.) etc. can be used.

[0017]When extracting the JINSENO sides in this invention from a crude drug, it does not limit to this, but it is preferred to extract from a ginseng or Ginseng Radix Rubra for example. a ginseng (ginseng radix: Ginseng Radix) means what passed lightly the root or this except the

rootlet of *Panax schinseng* through hot water, and is independent about those portions -- it is -- it can be used, combining arbitrarily. *Ginseng Radix Rubra* (*red ginseng; Red Ginseng; Ginseng Radix Rubra*) means what steamed the root of *Panax schinseng*, and is independent about those portions -- it is -- it can be used, combining arbitrarily.

[0018]The ginseng extract or the Ginseng Radix Rubra extract which can be used as an active principle in synthetic inhibitor by this invention should just contain the aforementioned JINSENO sides, especially JINSENO side Rg₁, therefore the crude extract of a ginseng or

Ginseng Radix Rubra can be used for it. It can obtain by water's (for example, chilled water's, warm water's, preferably boiling water's) extracting a ginseng or Ginseng Radix Rubra, or extracting it using an organic solvent as a manufacturing method of the ginseng extract which can be used by this invention, or the Ginseng Radix Rubra extract. as an organic solvent -- alcohol (for example, methyl alcohol.) of the carbon numbers 1-6 Ethyl alcohol, n-propyl alcohol, isopropyl alcohol, or butyl alcohol and ester (for example, methyl acetate and ethyl acetate.) Propyl acetate or butyl acetate, ketone (for example, acetone or methyl isobutyl ketone), Ether, petroleum ether, n-hexane, cyclohexane, toluene, the halogen derivative (for example, a carbon tetrachloride and dichloromethane.) of benzene and hydrocarbon or chloroform, pyridine, and a glycol (for example, propylene glycol.) Or a butylene glycol, a polyethylene glycol, or acetonitrile can be used, and these organic solvents can be mixed by independence or proper combination, and a fixed ratio, and also it can use by anhydrous or a moisture state. Preferably, methyl alcohol etc. are desirable. Being able to use the method used for the usual crude drug extraction as the method of water extraction or organic solvent extraction, for example, stirring to ginseng (desiccation) or Ginseng Radix Rubra 1 weight section using three to water or organic solvent 300 weight section. It is desirable at the temperature below the boiling point ultrasonic extraction or to carry out maceration at heating flowing back and ordinary temperature. The extraction process can shorten extraction time by carrying out preferably for 10 minutes - 24 hours, and usually adding supplementary means, such as stirring, for 5 minutes - seven days, if needed.

[0019] After the end of an extraction process, by suitable methods, such as filtration or centrifugal separation, an insoluble matter can be separated and a crude extract can be obtained from water or an organic solvent extract. In synthetic inhibitor of this invention, it may be used as it is, without refining especially the aforementioned crude extract, in using extraction, the JINSENO sides which carried out fractionation, especially JINSENO side Rg₁, from a natural product. The refining extracts processed further can also use the aforementioned crude extract other than the water extract by a conventional method, or an organic solvent extract as an active principle of synthetic inhibitor of this invention with various organic solvents or adsorbent. The ginseng extract or the Ginseng Radix Rubra extract containing the refining extracts which finished such crude extract and various kinds of

purification treatment. Even if it uses the extracted solution, the extract which condensed the solvent may be used, and the powder which distilled off the solvent and was dried and also the thing crystallized and refined, or a viscous substance may be used, and those diluents can also be used. In this way, the obtained ginseng extract or the Ginseng Radix Rubra extract contains the impurity which originates in the ginseng or Ginseng Radix Rubra of a raw material simultaneously, including as a mixture the JINSENO sides contained in a ginseng or Ginseng Radix Rubra.

[0020]The vegetable extract in which synthetic inhibitor of this invention contains JINSENO sides or JINSENO sides, for example, the extract of the crude drug containing JINSENO sides, (especially) about a ginseng extract or the Ginseng Radix Rubra extract, it is independent [its] -- or -- desirable -- galenical pharmacy -- the usual carrier permissible-like or in veterinary medicine -- an animal -- mammalian (especially Homo sapiens) can be medicated preferably. Especially as an administration pharmaceutical form, there is no limitation and For example, powder medicine, subtle granules, a granule, Parenterals, such as oral agents, such as a tablet, a capsule, suspension, an emulsion agent, syrups, extracts, or a pill, or injections, liquids for external use, an ointment, suppositories, cream of local administration, or eye drops, can be mentioned. These oral agents, for example Gelatin, sodium alginate, starch, Cornstarch, white soft sugar, milk sugar, grape sugar, mannite, carboxymethyl cellulose, Dextrin, a polyvinyl pyrrolidone, crystalline cellulose, a soybean lecithin, Sucrose, fatty acid ester, talc, magnesium stearate, a polyethylene glycol, In accordance with a conventional method, it can manufacture using excipients, such as a magnesium silicate, a silicic acid anhydride, or synthetic aluminum silicate, a binding material, disintegrator, a surface-active agent, lubricant, a fluid accelerator, a diluent, a preservative, colorant, perfume, corrigent, a stabilizing agent, a moisturizer, an antiseptic, or an antioxidant. For example, it is the capsule etc. which were mixed and filled up with JINSENO side Rg₁ of one weight section, and milk sugar of 99 weight sections.

[0021]As the parenteral administration method, injection (inside of hypodermic and a vein, etc.) or rectum administration is illustrated. In these, injections are used most suitably. In preparation of injections, for example, the JINSENO sides (especially JINSENO side Rg₁) as an active principle. (Or the extract of the vegetation containing JINSENO sides, for example, the extract of the crude drug containing JINSENO sides, (especially)) Besides a ginseng extract or the Ginseng Radix Rubra extract, for example Water soluble solvents, such as a physiological saline or Ringer's solution, Isotonizing agents, such as nonaqueous solubility solvents, such as vegetable oil or fatty acid ester, grape sugar, or sodium chloride, a solubilizing agent, a stabilizing agent, an antiseptic, a suspending agent, or an emulsifier can be used arbitrarily. Synthetic inhibitor of this invention may be prescribed for the patient using the technique of a sustained release drug in which the controlled-release polymer etc. were

used. For example, synthetic inhibitor of this invention can be made to be able to incorporate into the pellet of ethylene vinyl polymer acetate, and it can transplant surgically during the organization which should treat this pellet.

[0022] Although synthetic inhibitor of this invention is not limited to this, it can contain JINSENO sides in 0.1 to 80 weight % of quantity preferably 0.01 to 99 weight %. (The extract of the vegetation containing JINSENO sides, for example, the extract of the crude drug containing JINSENO sides, (especially)) Synthetic inhibitor of this invention which contains a ginseng extract or the Ginseng Radix Rubra extract as an active principle can be suitably adjusted so that the JINSENO sides (especially JINSENO side Rg₁) contained in it may become the aforementioned quantity range, and it can be prepared. (The extract of the vegetation containing JINSENO sides, for example, the extract of the crude drug containing JINSENO sides, (especially)) It is preferred to pharmaceutical-preparation-ize it using a carrier permissible in galenical pharmacy, in considering the synthetic inhibitor which contains a ginseng extract or the Ginseng Radix Rubra extract as an active principle as the pharmaceutical preparation for internal use.

[0023] The dose in the case of using synthetic inhibitor of this invention, Although it changes with a sick kind, a patient's age, sex, weight, the grade of condition, or medication methods and there is no restriction in particular, about 1mg-10g is usually prescribed [as an amount of JINSENO side Rg₁] for the patient in taking orally or parenterally in about 1 to 4 steps per day per adult. It is also possible for a use not to be limited to drugs, either and to give it in the form of ingesta as various uses, for example, functional food, and health food.

[0024]

[Function] As described above, the JINSENO sides contained in synthetic inhibitor of this invention, especially JINSENO side Rg₁, Since there is an operation which controls HSP47 intracellular composition specifically, if said JINSENO sides are prescribed for the patient, HSP47 intracellular biosynthesis will decrease specifically and the biosynthesis of collagen will be controlled. As a result, extracellular matrix production will also be controlled. Therefore, the illness which shows the symptoms of the extracellular-matrix-production sthenia accompanied by the increase in collagen in said JINSENO sides, For example, it can be used for the prevention and the therapy of keloid, a hypertrophic scar, a scleroderma, arteriosclerosis, or articular rheumatism which are produced after liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ***** disease), cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc. That is, synthetic inhibitor of this invention controls composition of collagen by controlling composition of HSP47 which is a collagen specific chaperon.

[0025] Since playing a role with important collagen synthesis in basement membrane and

basement membrane is pointed out, also in the vascularization, as mentioned above synthetic inhibitor of this invention, Are very useful as a prevention remedy of many diseases based on the plague of the vascularization, Each disease which was described previously, i.e., diabetic retinopathy, back fibrae-lentis vegetation, It can use for the vascularization accompanying a corneal transplantation, glaucoma, an eye tumor, trachoma, the pyogenic granuloma that cannot be dried, hemangioma, angioma fibrosum, a tumescent scar, granulation, rheumatism nature arthritis, the scleredema, atherosclerosis, various tumors, etc. Since it is clear to achieve a guiding role until the cancer cell in which the stromata (interstitial stroma) made into a basic skeleton furthermore seceded from I-beam collagen and fibronectin in metastasis of cancer invades into a nearby vessel ["BIOThERAPY", 7(8) : 1181, 1993] By prescribing synthetic inhibitor of this invention for the patient, it is also possible to control metastasis of cancer.

[0026]

[Working example]Hereafter, although an embodiment explains this invention concretely, these do not limit the range of this invention.

Embodiment 1: Peptide which consists of 15 amino acid corresponding to the amino terminal of preparation Homo sapiens HSP47 of the production (1) anti-HSP47 polyclonal antibody of anti-HSP47 polyclonal antibody to the 2-16th amino acid sequences It produces using the automatic peptide synthesis equipment (PSSM-8 system, the Shimazu work place) [hereafter called Homo sapiens HSP47 peptide (2-16)], and is SUKUSHINIMIJIRU 4-(p-maleimide phenyl) butyrate. Using [SMPB:Succinimidyl 4-(p-maleimidophenyl) butyrate] as a cross linking agent, it was made to combine with lactoglobulin with a conventional method ("Biochemistry", 18:690, 1979), and the sensitized antigen was produced. Phosphate buffered saline containing this sensitized-antigen 150mug [presentation: :Cosmobio which calls below KCl=0.2 g/l, KH₂PO₄=0.2 g/l, NaCl=8 g/l, and Na₂HPO₄(anhydrous)=1.15-g/l:PBS (-), catalog number 320-

01] The hypodermic of the roux rat (6-week old, feminity: CLEA Japan) was medicated with 0.2 ml of mixed liquor obtained by mixing 0.2 ml and equivalent weight of Freund's complete adjuvant (YATORON, catalog number RM606-1), and immunity was carried out. After repeating second and the 3rd immunity in a similar way, immunization was performed 6 times using the adjuvant (Hunter's TiterMax; CytRx Corporation, Georgia, U.S.). It collected blood from the sensitization animal, and the blood serum was separated with the conventional method, it extracted, and the antibody titer in a blood serum was measured by the enzyme-labeled antibody technique (the ELISA method) and Western blot technique which are shown below.

[0027](2) Homo sapiens HSP47 peptide (2-16) prepared for the evaluation preceding clause (1) of the anti-HSP47 polyclonal-antibody characteristic by an enzyme-labeled antibody technique (the ELISA method) is dissolved in PBS (-), The peptide solution of 10 microg/ml

concentration was prepared, and said peptide solution was dropped at each well of the rigid ASEI plate (a falcon, the catalog number 3910) every [50micro / l]. After putting only 50micro of PBS(-) l into the outermost well and neglecting it at 4 ** under humid overnight, After throwing away said peptide solution and washing each well using PBS (-), 100micro of PBS(-) l which contains bovine serum albumin (it is hereafter called BSA for short) 1% was put into each well, and it was neglected under the room temperature for 1 hour. After washing 3 times by PBS (-), roux rat blood serum 50mul acquired for the preceding clause (1) was put into each well, and it allowed to stand at the room temperature for 1 hour. After washing 3 times by PBS (-), peroxidase-labeling anti-rat IgG50microl was put into each well as a secondary antibody, and it was neglected under the room temperature for 1 hour. After washing twice by PBS (-), The substrate liquid every l [100mu] which dissolved and prepared one o-phenylenediamine (OPD) tablet (a sigma, the catalog number P8287) (10 mg) to 10 ml of 0.1M citrate buffers (pH 4.5) which added hydrogen-peroxide-solution 4mul is dropped at each well, After allowing to stand for 30 minutes under protection from light at a room temperature, the absorbance of 492 nm of each well was measured with the microplate reader (TOSOH, MPR-A4i type). The blood serum with which the rise of antibody titer was checked was used as the anti human HSP47 polyclonal antibody, and was used for the following embodiments.

[0028](3) The buffer system (Laemmli, N. K., "Nature", 283:pp. 249-256, 1970) of the evaluation Laemmli of the anti-HSP47 polyclonal-antibody characteristic by a Western blot technique is used, Sodium-dodecyl-sulfate (SDS) polyacrylamide gel electrophoresis of the lysate of a HeLa cell was performed in accordance with the following methods. Preparation of concentrating gel was performed as follows. 6.1 ml of distilled water, 0.5M tris (Bio-Rad, catalog number 161-0716) - HCl(pH 6.8) 2.5ml, 10% SDS(Bio-Rad, catalog number 161-0301) 100microl, And 1.3 ml of 30% acrylamide (Bio-Rad, catalog number 161-0101) / N,N'-methylenebis acrylamide (Bio-Rad, catalog number 161-0201) are mixed, Carry out indirect desulfurization mind for 15 minutes, and 10% ammonium persulfate (Bio-Rad, catalog number 161-0700) 50microl and N,N,N',N'-tetramethylethylenediamine. (It is hereafter called TEMED for short) 10microl -- in addition, concentrating gel was prepared (Bio-Rad, catalog number 161-0800). Preparation of resolving gel was performed as follows. 4.045 ml of distilled water, 1.5M tris-HCl(pH 8.8) 2.5ml, 10% SDS100microl, and 3.3 ml of 30% acrylamide / N,N'-methylenebis acrylamide are mixed slowly, It degassed with the aspirator for 15 minutes, and 10% ammonium persulfate 50mul and TEMED5microl were added. As a migration buffer, distilled water was added to 9.0 g of tris, 43.2 g of glycines (Bio-Rad, catalog number 161-0717), and SDS3.0g, it was made 600 ml, and what diluted this with distilled water 5 times was used. A sample buffer 2 ml of distilled water, 2M tris-HCl(pH 6.8) 500microl, What mixed SDS0.32g and beta-mercaptoethanol 800microl and 0.05% (w/v) bromophenol-blue (Bio-Rad, catalog number 161-0404) 400microl was used.

[0029]Under 5% carbon dioxide conditions, at 37 **, the HeLa cell was cultured in the MEM culture medium which contains inactivation fetal calf serum (it is hereafter called FBS for short) 10%, and the lysate was prepared. After performing SDS-polyacrylamide gel electrophoresis of the obtained HeLa-cell lysate, Gel is stuck to a 0.45-micrometer nitrocellulose membrane (Schleicher & Schuell, catalog number 401196), The room temperature performed blotting by 100V for 3 hours using protein transfer equipment (Trans-Blot Electrophoretic Transfer Cell: Bio-Rad). As a blotting buffer. ** 0.025M tris. And the trisglycine buffer which consisted of 0.192M glycine and was adjusted the pH to 8.5 () [Tris Gly Running and Blotting Buffer;Enprotech and] The U.S. Massachusetts state and the buffer which prepared methyl alcohol in addition to catalog number SA100034 so that it might become 20% were used. It blocked by dipping a nitrocellulose membrane in the PBS (-) solution which contains skim milk (Snow Brand Milk Products) 5% for 30 minutes at a room temperature after blotting. Primary antibody reactions were performed after blocking using the screener blotter (sampler tech) by making into a primary antibody the roux rat blood serum acquired for the preceding clause (1). It is said roux rat blood serum 200mul diluted with PBS (-) which contains skim milk (Snow Brand Milk Products) 2% 10 times, and primary antibody reactions were performed for 120 minutes at the room temperature. The shake for 5 minutes by PBS (-) after primary antibody ending reaction using a slow locking shaker 2 times, The nitrocellulose membrane was washed by the PBS (-) solution which contains Tween20 (Bio-Rad, catalog number 170-6531) 0.1% performing shake for 15 minutes, and performing shake for 5 minutes twice by 4 times and also PBS (-). Secondary antibody reactions were performed after the end of washing for 2 hours using 5 ml of solutions which diluted the peroxidase-labeling goat anti-rat IgG antibody (Southern Biotechnology, catalog number 3030-05) with the PBS (-) solution which contains skim milk 2% 5000 times. The PBS (-) solution and the PBS (-) solution which contains Tween20 0.1% washed the nitrocellulose membrane under the same conditions as washing after primary antibody reactions after ending reaction.

[0030]After removing an excessive PBS (-) solution, a Western-blotting detecting reagent (ECL Western blotting detection reagent; Amersham, catalog number RPN2106) is sprinkled on a nitrocellulose membrane, After settling at a room temperature for 1 minute, an excessive detecting reagent is removed, a nitrocellulose membrane is wrapped in a lap, and a reaction surface was stuck to an X-ray film (Kodak X-OMAT, AR, catalog number 165 1454), and was made to expose. Examination of the reactivity of anti-HSP47 polyclonal antibody was performed after development by measuring a band near [equivalent to HSP47] a molecular weight of 47 kilodalton. A blood serum with which a rise of antibody titer was checked was used for the following embodiments as anti human HSP47 polyclonal antibody.

[0031]Embodiment 2 : culture human breast cancer cell stock MCF7 (ATCC HTB 22) of a measurement (1) Homo sapiens cultured cancer cell of a HSP expression amount of the Homo

sapiens cultured cancer cell, In RPMI1640 culture medium including 10^{-8} Mbeta-estradiol and the 10% inactivation FBS, it cultivated at 37 ** under 5% carbon dioxide conditions except the time of heat shock processing.

[0032](2) JINSENO side Rg₁ (Matsuura Yakugyo) expressed with said formula (I) that it becomes the last concentration M of 100micro in a culture medium of said human breast cancer cell stock MCF7 two days after JINSENO side Rg₁ processing and heat shock processing seeding was added, and it cultivated for 24 hours. Then, after carrying out heat shock processing for 15 minutes at 45 **, it cultivated at 37 ** all night. A control test was carried out like the above except not adding JINSENO side Rg₁.

[0033](3) Each cell processed for the measurement preceding clause (2) of the HSP expression amount in the Homo sapiens cultured cancer cell was homogenized by the method shown below, and the HSP expression amount was measured in the Western blot technique. Namely, the lysis buffer (lysis buffer) after washing the cell processed for the preceding clause (2) by PBS (-) [1.0%NP-40, 0.15M sodium chloride, the 50mM tris- HCl (pH 8.0), 5 mM-EDTA, 2 mM-N-ethylmaleimide, 2mM phenylmethyl sulfonylfluoride, 2 microg [/ml] leupeptin, and 2 microg [/ml] pepstatin] 1 ml was added and it settled for 20 minutes in Hikami. Then, centrifugality was performed for 20 minutes at 12000 rpm at 4 **. 10micro of supernatant liquid I after centrifugality was added to 790micro of PBS(-) I, and also 200micro of protein assay stain solutions (Dye Reagent Concentrate : Bio-Rad, catalog number 500-0006) I were added. For 5 minutes, after settling at a room temperature, the absorbance was measured at 595 nm and protein quantification was performed. The SDS polyacrylamide gel electrophoresis of the lysate which contains equivalent weight of protein by the buffer system of Laemmli was performed using the sample which performed protein quantification. In accordance with the method described in Embodiment 1, blocking following blotting and it was performed after the electrophoresis. Namely, protein transfer equipment (Trans-Blot Electrophoretic Transfer Cell: Bio-Rad) is used, At the room temperature, gel was stuck to a 0.45-micrometer nitrocellulose membrane (Schleicher & Schuell, catalog number 401196), and blotting was performed 100V for 3 hours. As a blotting buffer, the same thing as the buffer used in said Embodiment 1 (3) was used. After blotting, the nitrocellulose membrane was incubated for 30 minutes at the room temperature in the 10% skim milk (Snow Brand Milk Products)-PBS (-) solution, and nonspecific combination was blocked.

[0034]An anti human HSP47 rat polyclonal antibody manufactured in Embodiment 1 performed primary antibody reactions on a nitrocellulose membrane after blocking. Then, exchange every [a for / 5 minutes], and a solution by PBS (-), and a slow locking shaker performs two washing, Every [a for / 15 minutes] and a solution were exchanged with Tween20 (Bio-Rad, catalog number 170-6531) solution PBS(-)-0.1%, and four washing was performed. Eventually,

every [a for / 5 minutes] two washing was performed by PBS (-). A peroxidase-labeling goat anti-rat IgG antibody (Southern Biotechnology, catalog number 3030-05) after an end of washing, Secondary antibody reactions were performed for 2 hours using 5 ml of antibody solutions diluted and prepared 5000 times with an PBS (-) solution which contains skim milk 2%. After ending reaction, about a nitrocellulose membrane, a solution was changed for 5 minutes at a time with an PBS (-) solution, a solution was changed for 15 minutes at a time with Tween20 solution 2 times and also PBS(-)-0.1%, and a slow locking shaker performed five washing. Finally an PBS (-) solution performed every [a for / 5 minutes] two washing. After removing an excessive PBS (-) solution, a Western-blotting detecting reagent (ECL Westernblotting detection reagent;Amersham and catalog number RPN2106) is sprinkled on a nitrocellulose membrane, After incubating for 1 minute, an excessive detecting reagent was removed, a nitrocellulose membrane was wrapped in a lap, a reaction surface was stuck to an X-ray film (Kodak X-OMAT, AR, catalog number 165 1454), was exposed, was developed, and existence of HSP47 was examined.

[0035]In breast cancer cell line MCF7 which did not add a control test, i.e., JINSENO side Rg₁, the band of molecular weight abbreviation 47kD was detected one. In breast cancer cell line MCF7 which added JINSENO side Rg₁, the corresponding band was a very thin band. That is, JINSENO side Rg₁ can be concluded to have the activity of the synthetic inhibitor which controls the manifestation of HSP47, and this fact shows that JINSENO side Rg₁ works restrainedly to sthenia of extracellular matrix production.

[0036]

[Effect of the Invention]As explained in full detail above, synthetic inhibitor of this invention, For example, liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ***** disease), It has the operation which improves the collagen synthesis sthenia seen by the cell which shows the symptoms of sthenia of extracellular matrix production, such as keloid produced after cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc., a hypertrophic scar, a scleroderma, arteriosclerosis, or articular rheumatism, and which fell ill sick. Therefore, by prescribing synthetic inhibitor by this invention for the patient, an organ, the fibrosis of an organization, and hardening can be prevented, as a result, said sick patient's physiological status can be made to be able to improve effectively, and said illness can be treated effectively. Synthetic inhibitor of this invention is useful also for the prevention therapy of the various diseases accompanied by the plague of the vascularization. Since it is clear to achieve a guiding role until the cancer cell in which the stromata made into a basic skeleton seceded from I-beam collagen and fibronectin in metastasis of cancer invades into a nearby vessel, it is also possible by prescribing synthetic inhibitor of this invention for the patient to control metastasis of cancer.

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PRIOR ART

[Description of the Prior Art] In recent years, the illness which shows the symptoms of sthenia of production of extra-cellular matrices, such as collagen, poses a big problem. With the illness which shows the symptoms of sthenia of extracellular matrix production here. For example, keloid, a hypertrophic scar, a scleroderma, arteriosclerosis, or articular rheumatism produced after liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ***** disease), cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc. is included.

[0003] For example, the liver cirrhosis said for the deceased to climb at least our country also in about 20,000 people per year is the hepatic fibrosis of ***** covering the flume crack which is a sick general term which becomes hard since liver is growth of connective tissue, and is an end image of various chronic liver disease, and the whole liver. That is, in the chronic hepatitis which attains to a long period of time, liver injury, such as inflammation, carries out fibrosis of the liver with remarkable sthenia of extra-cellular-matrix (especially I-beam collagen) production of fibroblast, Ito cells, etc. If the fibrosis of a liver advances chronically, increasingly normal liver regeneration is blocked, hepatocytes are replaced, and the extra-cellular matrix which makes I-beam collagen fibroblast with a subject will occupy most portion of a hepatic tissue, and will result in the liver cirrhosis which consists of many *****. The abnormalities of the blood flow which a fiber septum progresses to the whole liver and produces as a result with advance of liver cirrhosis, It also becomes a cause which pushes the denaturation of a hepatocyte further, and the vicious circle in liver cirrhosis will continue, and also a lot of colloid fibers in liver are generated by various causes, such as alcohol, a virus, and autoimmunity, a necrosis and afunction of hepatocytes arise by them, and a cirrhotic patient dies finally. Although I-beam collagen occupies about 2% of a total protein amount in a normal liver, if it serves as liver cirrhosis, it will come to occupy 10 to 30%.

[0004] An interstitial lung disease is a disease group characterized by the chronic inflammation

(alveolitis alveolitis) of an alveolus and not only an alveolar duct but the lower respiratory tract which also often involves in respiratory bronchiole and an end bronchial tube, the fibrosis of the stromata which are the result, and alveolar endogenous-fibers-ization. With an interstitial lung disease here, diffusion interstitial lung diseases, such as pneumonitis and fibroid lung, idiopathic pulmonary fibrosis, the penetrable pulmonary edema, a collagen disease of lung, sarcoidosis, etc. are included, for example. In an interstitial lung disease, superfluous production and accumulation of the extra-cellular matrix are accepted in the fibrosis organization. That is, in the lung fibrosis organization of an interstitial lung disease, accumulation of a Tsuguaki I-beam and III type collagen is seen by enlarged stromata, the alveolar septa which carried out thickening to the early stage of fibrosis are piled up, a stadium reaches an advanced stage, I-beam collagen increases in the second half, and especially III type collagen turns into main collagen. Basement membrane is destroyed at an early stage, and invasion of the collagen fiber by the side of an alveolar space is observed.

[0005]Chronic renal failure is in the state where it became impossible to maintain homeostasis in the living body by desolation of a kidney function, as a result of chronic nephritic syndrome. When advance of chronic renal failure is seen pathologically, it is advance of glomerulosclerosis and stromata fibrosis. The glomerulosclerosis is the hyperplasia of the extra-cellular matrix centering on a mesangium field. It compares that it is normal, and the ingredient of glomerular basement membrane, such as IV type collagen, increases to Tsuguaki, and the ingredient of mesangium sclerosis is carrying out hyperplasia also of the I-beam collagen which is a stromata ingredient in accordance with the sclerosis part. That is, it is a factor with major production sthenia of an extra-cellular matrix to the glomerulosclerosis which passes in chronicity. With a ***** disease, IgA glomerulonephritis, focal glomerular sclerosis, membranoproliferative glomerulonephritis, diabetic nephropathy, a chronic interstitial nephritis, chronic glomerulonephritis, etc. are included in chronic renal failure here, for example.

[0006]A cardiac muscle cell is a cell which specialized highly, and does not have the capability with it to divide and increase. Therefore, if a certain load is added to the heart, the each will get fat, and a cardiac muscle cell will increase a shrinkage force, and will try to maintain cardiac performance. If load continues for a long time, a variegated obstacle will be accumulated focusing on the factor of the ischemia, cause a breakdown to the compensatory mechanism over load, the shrinkage force of a myocardium declines rapidly, and the pumping ability of the heart is spoiled -- cardiac insufficiency -- ***** -- things are known and cardiac hypertrophy occupies the biggest portion as the origin of the cardiac insufficiency in our country. The merger rate of ischemic heart disease or critical ventricular arrhythmia becomes high intentionally, and formation of cardiac hypertrophy not only becomes the greatest risk factor of the onset of cardiac insufficiency, but has become a factor which specifies a vital prognosis

independently. In order that each cardiac muscle cell not only gets fat remarkably, but may bundle the cardiac muscle cell firmly at the time of cardiac hypertrophy progress, the fibrosis of stromata is promoted and collagen which is an extra-cellular matrix increases. If a cardiac muscle cell is lost by myocarditis, myocardial ischemia, etc., collagen will be biosynthesized and a gap will be replaced. If the fibrosis of stromata progresses superfluously as a result, a myocardium will become hard and the obstacle of the extension will be carried out. A muscle snug function also falls and the obstacle also of the relaxation of the diastole of a myocardium is carried out. In addition, the gastric upset of collagen synthesis happens by a certain cause, and the illnesses which show the symptoms of extracellular-matrix-production sthenia of a postoperative scar, a burn nature scar or a scleroderma, arteriosclerosis, etc. are considered to be the origins with main fibrosis progressing and producing hardening change of an organization.

[0007]Also in the vascularization, the collagen synthesis in basement membrane and basement membrane, An important role. Achieving is pointed out (). [Maragoudakis and] E., Sarmonika, M., and and. Panoutsacopoulous and M., "J. Pharmacol. Exp. Ther.", 244: 729, 1988 ; Ingber, D. E., Madri, J. A., and Folkman, J., "Endocrinology", 119: 1768, 1986. As a disease by the vascularization, for example Diabetic retinopathy, back fibrae-lentis vegetation, The vascularization accompanying a corneal transplantation, glaucoma, an eye tumor, trachoma, a trunk plug, the pyogenic granuloma, hemangioma, angioma fibrosum, a tumescent scar, granulation, rheumatism nature arthritis, the scleredema, atherosclerosis, various tumors, etc. are known. Thus, although the illness which shows the symptoms of sthenia of production of extra-cellular matrices, such as collagen, poses a big problem, In the former, extra-cellular-matrix synthetic inhibitor (for example, collagen synthesis depressant) should be satisfied with various fields, such as side effects and a medicinal value, of synthetic inhibitor was not yet developed.

[0008]On the other hand, a heat shock protein (it is also called heat shock protein;HSP and stress protein), a group revealed by the cell by stimulating a cell by a certain stress, for example, heat, a heavy metal, drugs, an amino acid analog, or hypoxia (low concentration oxygen) -- it is protein. The heat shock protein exists in the nature universally.

It is produced by bacteria, yeast, vegetation, an insect, and the higher animal including Homo sapiens.

Although the kind is various, HSP, From the size of a molecular weight to HSP90 family (for example, HSP of 90kD or 110kD, etc.). It can divide roughly into 4 of HSP70 family, HSP(s)(for example, HSP of 70 - 73kD, etc.)60 family, and low molecule HSP(s) (for example, HSP of 57 - 68kD, etc.) families (for example, HSP of 20kD, 25 - 28kD, or 47kD, etc.) families. In this Description, the number indicated to be HSP immediately after that shall show HSP which has specific molecular weight, for example, HSP of molecular weight 47kD shall be called

"HSP47." As mentioned above, although many kinds exist in HSP, these differ not only in a molecular weight but in structure, a function, or character etc., respectively. the response to stress -- in addition, some of these protein being compounded compositionally and under normal environment, A thing like a meeting of proteinic folding, unfolding, and a protein subunit and proteinic membrane transport for which the indispensable physiological role is played is shown. These functions as a heat shock protein are called a molecular chaperone.

[0009]HSP47 is the protein discovered by Hitoshi Nagata in 1986, and is basic protein ($\text{pI}=9.0$) with a molecular weight of 47 kilodalton. As the manifestation of HSP47 increases, It is shown by various cells that composition of collagen also increases ("J. Biol. Chem." 261: 7531 and 1986; " Eur. J. Biochem." 206:323 and 1992; " J.). Biol. Chem.", 265 : 992, 1990 ; "J. Clin. Invest.", 94:2481, 1994. Namely, HSP47 is an aspect of affairs of processing of the procollagen within an endoplasmic reticulum, 3 heavy-chain helix formation, or the procollagen transportation and secretion to Golgi apparatus from an endoplasmic reticulum in intracellular, Since it is functioning as a specific molecular chaperone of collagen, the HSP47 manifestation which increased stimulates accumulation of the collagen molecule in an extra-cellular matrix. Thus, HSP47 which is a collagen binding heat shock protein is the heat shock protein which related to collagen which is extracellular matrix protein closely also in the function like the manifestation.

[Translation done.]

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EXAMPLE

[Working example] Hereafter, although an embodiment explains this invention concretely, these do not limit the range of this invention.

Embodiment 1: Peptide which consists of 15 amino acid corresponding to the amino terminal of preparation Homo sapiens HSP47 of the production (1) anti-HSP47 polyclonal antibody of anti-HSP47 polyclonal antibody to the 2-16th amino acid sequences It produces using the automatic peptide synthesis equipment (PSSM-8 system, the Shimazu work place) [hereafter called Homo sapiens HSP47 peptide (2-16)], and is SUKUSHINIMIJIRU 4-(p-maleimide phenyl) butyrate. Using [SMPB:Succinimidyl 4-(p-maleimidophenyl) butyrate] as a cross linking agent, it was made to combine with lactoglobulin with a conventional method ("Biochemistry", 18:690, 1979), and the sensitized antigen was produced. Phosphate buffered saline containing this sensitized-antigen 150mug [presentation: :Cosmobio which calls below KCl=0.2 g/l, KH₂PO₄=0.2 g/l, NaCl=8 g/l, and Na₂HPO₄(anhydrous)=1.15-g/l:PBS (-), catalog number 320-01] The hypodermic of the roux rat (6-week old, femininity: CLEA Japan) was medicated with 0.2 ml of mixed liquor obtained by mixing 0.2 ml and equivalent weight of Freund's complete adjuvant (YATORON, catalog number RM606-1), and immunity was carried out. After repeating second and the 3rd immunity in a similar way, immunization was performed 6 times using the adjuvant (Hunter's TiterMax; CytRx Corporation, Georgia, U.S.). It collected blood from the sensitization animal, and the blood serum was separated with the conventional method, it extracted, and the antibody titer in a blood serum was measured by the enzyme-labeled antibody technique (the ELISA method) and Western blot technique which are shown below.

[0027](2) Homo sapiens HSP47 peptide (2-16) prepared for the evaluation preceding clause (1) of the anti-HSP47 polyclonal-antibody characteristic by an enzyme-labeled antibody technique (the ELISA method) is dissolved in PBS (-), The peptide solution of 10 microg/ml concentration was prepared, and said peptide solution was dropped at each well of the rigid

ASEI plate (a falcon, the catalog number 3910) every [50micro / 1]. After putting only 50micro of PBS(-) I into the outermost well and neglecting it at 4 ** by humid Shimo overnight, After throwing away said peptide solution and washing each well using PBS (-), 100micro of PBS(-) I which contains bovine serum albumin (it is hereafter called BSA for short) 1% was put into each well, and it was neglected under the room temperature for 1 hour. After washing 3 times by PBS (-), roux rat blood serum 50mul acquired for the preceding clause (1) was put into each well, and it allowed to stand at the room temperature for 1 hour. After washing 3 times by PBS (-), peroxidase-labeling anti-rat IgG50microl was put into each well as a secondary antibody, and it was neglected under the room temperature for 1 hour. After washing twice by PBS (-), The substrate liquid every 1 [100mu] which dissolved and prepared one o-phenylenediamine (OPD) tablet (a sigma, the catalog number P8287) (10 mg) to 10 ml of 0.1M citrate buffers (pH 4.5) which added hydrogen-peroxide-solution 4mul is dropped at each well, After allowing to stand for 30 minutes under protection from light at a room temperature, the absorbance of 492 nm of each well was measured with the microplate reader (TOSOH, MPR-A4i type). The blood serum with which the rise of antibody titer was checked was used as the anti human HSP47 polyclonal antibody, and was used for the following embodiments.

[0028](3) The buffer system (Laemmli, N. K., "Nature", 283:pp. 249-256, 1970) of the evaluation Laemmli of the anti-HSP47 polyclonal-antibody characteristic by a Western blot technique is used, Sodium-dodecyl-sulfate (SDS) polyacrylamide gel electrophoresis of the lysate of a HeLa cell was performed in accordance with the following methods. Preparation of concentrating gel was performed as follows. 6.1 ml of distilled water, 0.5M tris (Bio-Rad, catalog number 161-0716) - HCl(pH 6.8) 2.5ml, 10% SDS(Bio-Rad, catalog number 161-0301) 100microl, And 1.3 ml of 30% acrylamide (Bio-Rad, catalog number 161-0101) / N,N'-methylenebis acrylamide (Bio-Rad, catalog number 161-0201) are mixed, Carry out indirect desulfurization mind for 15 minutes, and 10% ammonium persulfate (Bio-Rad, catalog number 161-0700) 50microl and N,N,N',N'-tetramethylethylenediamine. (It is hereafter called TEMED for short) 10microl -- in addition, concentrating gel was prepared (Bio-Rad, catalog number 161-0800). Preparation of resolving gel was performed as follows. 4.045 ml of distilled water, 1.5M tris-HCl(pH 8.8) 2.5ml, 10% SDS100microl, and 3.3 ml of 30% acrylamide / N,N'-methylenebis acrylamide are mixed slowly, It degassed with the aspirator for 15 minutes, and 10% ammonium persulfate 50mul and TEMED5microl were added. As a migration buffer, distilled water was added to 9.0 g of tris, 43.2 g of glycines (Bio-Rad, catalog number 161-0717), and SDS3.0g, it was made 600 ml, and what diluted this with distilled water 5 times was used. A sample buffer 2 ml of distilled water, 2M tris-HCl(pH 6.8) 500microl, What mixed SDS0.32g and beta-mercaptoethanol 800microl and 0.05% (w/v) bromophenol-blue (Bio-Rad, catalog number 161-0404) 400microl was used.

[0029]Under 5% carbon dioxide conditions, at 37 **, the HeLa cell was cultured in the MEM

culture medium which contains inactivation fetal calf serum (it is hereafter called FBS for short) 10%, and the lysate was prepared. After performing SDS-polyacrylamide gel electrophoresis of the obtained HeLa-cell lysate, Gel is stuck to a 0.45-micrometer nitrocellulose membrane (Schleicher & Schuell, catalog number 401196), The room temperature performed blotting by 100V for 3 hours using protein transfer equipment (Trans-Blot Electrophoretic Transfer Cell: Bio-Rad). As a blotting buffer. ** 0.025M tris. And the trisglycine buffer which consisted of 0.192M glycine and was adjusted the pH to 8.5 () [Tris Gly Running and Blotting Buffer;Enprotech and] The U.S. Massachusetts state and the buffer which prepared methyl alcohol in addition to catalog number SA100034 so that it might become 20% were used. It blocked by dipping a nitrocellulose membrane in the PBS (-) solution which contains skim milk (Snow Brand Milk Products) 5% for 30 minutes at a room temperature after blotting. Primary antibody reactions were performed after blocking using the screener blotter (sampler tech) by making into a primary antibody the roux rat blood serum acquired for the preceding clause (1). It is said roux rat blood serum 200mul diluted with PBS (-) which contains skim milk (Snow Brand Milk Products) 2% 10 times, and primary antibody reactions were performed for 120 minutes at the room temperature. The shake for 5 minutes by PBS (-) after primary antibody ending reaction using a slow locking shaker 2 times, The nitrocellulose membrane was washed by the PBS (-) solution which contains Tween20 (Bio-Rad, catalog number 170-6531) 0.1% performing shake for 15 minutes, and performing shake for 5 minutes twice by 4 times and also PBS (-). Secondary antibody reactions were performed after the end of washing for 2 hours using 5 ml of solutions which diluted the peroxidase-labeling goat anti-rat IgG antibody (Southern Biotechnology, catalog number 3030-05) with the PBS (-) solution which contains skim milk 2% 5000 times. The PBS (-) solution and the PBS (-) solution which contains Tween20 0.1% washed the nitrocellulose membrane under the same conditions as washing after primary antibody reactions after ending reaction.

[0030]After removing an excessive PBS (-) solution, a Western-blotting detecting reagent (ECL Western blotting detection reagent; Amersham, catalog number RPN2106) is sprinkled on a nitrocellulose membrane, After settling at a room temperature for 1 minute, an excessive detecting reagent is removed, a nitrocellulose membrane is wrapped in a lap, and the reaction surface was stuck to the X-ray film (Kodak X-OMAT, AR, catalog number 165 1454), and was made to expose. Examination of the reactivity of anti-HSP47 polyclonal antibody was performed after development by measuring the band near [equivalent to HSP47] the molecular weight of 47 kilodalton. The blood serum with which the rise of antibody titer was checked was used for the following embodiments as anti human HSP47 polyclonal antibody.

[0031]Embodiment 2 : culture human breast cancer cell stock MCF7 (ATCC HTB 22) of the measurement (1) Homo sapiens cultured cancer cell of the HSP expression amount of the Homo sapiens cultured cancer cell, In the RPMI1640 culture medium including 10^{-8} Mbeta-

estradiol and the 10% inactivation FBS, it cultivated at 37 ** under 5% carbon dioxide conditions except the time of heat shock processing.

[0032](2) JINSENO side Rg₁ (Matsuura Yakugyo) expressed with said formula (I) that it becomes the last concentration M of 100micro in the culture medium of said human breast cancer cell stock MCF7 two days after JINSENO side Rg₁ processing and heat shock processing seeding was added, and it cultivated for 24 hours. Then, after carrying out heat shock processing for 15 minutes at 45 **, it cultivated at 37 ** all night. The control test was carried out like the above except not adding JINSENO side Rg₁.

[0033](3) Each cell processed for the measurement preceding clause (2) of the HSP expression amount in the Homo sapiens cultured cancer cell was homogenized by the method shown below, and the HSP expression amount was measured in the Western blot technique. Namely, the lysis buffer (lysis buffer) after washing the cell processed for the preceding clause (2) by PBS (-) [1.0%NP-40, 0.15M sodium chloride, the 50mM tris- HCl (pH 8.0), 5 mM-EDTA, 2 mM-N-ethylmaleimide, 2mM phenylmethyl sulfonylfluoride, 2 microg [/ml] leupeptin, and 2 microg [/ml] pepstatin] 1 ml was added and it settled for 20 minutes in Hikami. Then, centrifugality was performed for 20 minutes at 12000 rpm at 4 **. 10micro of supernatant liquid I after centrifugality was added to 790micro of PBS(-) I, and also 200micro of protein assay stain solutions (Dye Reagent Concentrate : Bio-Rad, catalog number 500-0006) I were added. For 5 minutes, after settling at a room temperature, the absorbance was measured at 595 nm and protein quantification was performed. The SDS polyacrylamide gel electrophoresis of the lysate which contains equivalent weight of protein by the buffer system of Laemmli was performed using the sample which performed protein quantification. In accordance with the method described in Embodiment 1, blocking following blotting and it was performed after the electrophoresis. Namely, protein transfer equipment (Trans-Blot Electrophoretic Transfer Cell: Bio-Rad) is used, At the room temperature, gel was stuck to a 0.45-micrometer nitrocellulose membrane (Schleicher & Schuell, catalog number 401196), and blotting was performed 100V for 3 hours. As a blotting buffer, the same thing as the buffer used in said Embodiment 1 (3) was used. After blotting, the nitrocellulose membrane was incubated for 30 minutes at the room temperature in the 10% skim milk (Snow Brand Milk Products)-PBS (-) solution, and nonspecific combination was blocked.

[0034]The anti human HSP47 rat polyclonal antibody manufactured in Embodiment 1 performed primary antibody reactions on the nitrocellulose membrane after blocking. Then, exchange every [a for / 5 minutes], and a solution by PBS (-), and a slow locking shaker performs two washing, Every [a for / 15 minutes] and a solution were exchanged with Tween20 (Bio-Rad, catalog number 170-6531) solution PBS(-)-0.1%, and four washing was performed. Eventually, every [a for / 5 minutes] two washing was performed by PBS (-). A

peroxidase-labelling goat anti-rat IgG antibody (Southern Biotechnology, catalog number 3030-05) after the end of washing, Secondary antibody reactions were performed for 2 hours using 5 ml of antibody solutions diluted and prepared 5000 times with the PBS (-) solution which contains skim milk 2%. After ending reaction, about the nitrocellulose membrane, the solution was changed for 5 minutes at a time with the PBS (-) solution, the solution was changed for 15 minutes at a time with Tween20 solution 2 times and also PBS(-)-0.1%, and the slow locking shaker performed five washing. Finally the PBS (-) solution performed every [a for / 5 minutes] two washing. After removing an excessive PBS (-) solution, a Western-blotting detecting reagent (ECL Westernblotting detection reagent;Amersham and catalog number RPN2106) is sprinkled on a nitrocellulose membrane, After incubating for 1 minute, the excessive detecting reagent was removed, the nitrocellulose membrane was wrapped in the lap, the reaction surface was stuck to the X-ray film (Kodak X-OMAT, AR, catalog number 165 1454), was exposed, was developed, and the existence of HSP47 was examined.

[0035]In breast cancer cell line MCF7 which did not add a control test, i.e., JINSENO side Rg₁, the band of molecular weight abbreviation 47kD was detected one. In breast cancer cell line MCF7 which added JINSENO side Rg₁, the corresponding band was a very thin band. That is, JINSENO side Rg₁ can be concluded to have the activity of the synthetic inhibitor which controls the manifestation of HSP47, and this fact shows that JINSENO side Rg₁ works restrainedly to sthenia of extracellular matrix production.

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CLAIMS**[Claim(s)]**

[Claim 1]Synthetic inhibitor of a heat shock protein with a molecular weight of 47 kilodalton containing JINSENO sides as an active principle.

[Claim 2]Synthetic inhibitor of a heat shock protein with an according to claim 1 molecular weight of 47 kilodalton whose JINSENO sides are JINSENO side Rg₁.

[Claim 3]Synthetic inhibitor of a heat shock protein with a molecular weight of 47 kilodalton containing an extract of vegetation containing JINSENO sides as an active principle.

[Claim 4]Synthetic inhibitor of a heat shock protein with a molecular weight of 47 kilodalton containing an extract of a ginseng or Ginseng Radix Rubra as an active principle.

[Translation done.]